Functional trait diversity of herb species in The Jena Experiment: Plastic responses or genetic variability?

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

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Zürich 2011

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General Introduction

Background

Human activities have caused rapid and continuous changes to the environment on local and global scale. These alterations lead to dramatic changes in the biotic structure and composition of communities (e.g. population size, distribution area), either due to the loss of species or to the introduction of new species. The consequences of biodiversity loss or changes in community composition for the functioning of ecosystems have been increasingly come into the focus of ecological research during the last decade.

Consequently, understanding the factors underlying the coexistence of many plant species at small spatial scale and processes of community assembly are a challenging issue in current ecological research. Plant community assembly is influenced by numerous interacting factors (Chesson 2000), including among others the diversity, quality and quantity of available resources (Petraitis et al. 1989; Chesson 2000; Kassen et al. 2000), trophic interactions such as the presence of herbivores and pathogens (Gallet et al. 2007; Meyer and Kassen 2007; Benmayor et al. 2008; Friman et al. 2008), disturbance (Petraitis et al. 1989; Roxburgh et al. 2004; Cadotte 2007) or phylogenetic history (Cavender-Bares et al. 2009). Different models predict how the distribution and abundances of species are determined as the neutral concept (Bell 2001; Hubbell 2001) and the classical concept of niche-based models (Hutchinson 1957; MacArthur and Levins 1967). The neutral theory of biodiversity and biogeography states that under the assumption that all species are equivalent to each other in all important ecological respects no single species has a competitive advantage or disadvantage. Therefore, species do not exclude each other (Bell 2000; Hubbell 2001, 2005), and stochasticity in species colonisation, extinction and speciation (MacArthur and Wilson 1963, 1967; Hubbell 2001; Volkov et al. 2003) explains community assembly and species coexistence. In contrast, the niche concept assumes that community dynamics are mainly evoked by differences in species' reproductive success and mortality. Based on differences in resource acquisition and use, species thereby avoid competition and are able to coexist (Hardin 1960; Chesson 2000). However, a separate niche-based and neutral model may be insufficient to describe community dynamics (Leibold and McPeek 2006). Both niche and neutral processes play interactive roles. Niche-based processes may be of minor importance when average fitness differences among species are small (Pielou 1978), i.e. in communities close to neutrality. Coexisting species exhibit processes that reduce both fitness differences and the relative intensity of interspecific competition. The environmental conditions under which individual species in a plant community must acquire essential resources such as water, carbon dioxide,

light and mineral nutrients depend on the characteristics of the surroundings and the interactions with neighbouring plants. Trade-offs in requirements for multiple resources enable numerous competitors to coexist and shape community structure (Bonsall et al. 2004; Tilman 2004; Gilbert et al. 2006). The greater the fitness differences between species, the stronger the stabilizing mechanisms need to be and vice versa (Chesson 2000). Stabilizing mechanisms themselves base on trade-offs of species functional characteristics (e.g. stronger competitors are weaker dispersers; Tilman 1994) or fluctuation-dependent mechanisms (e.g. the storage effect; Pacala and Tilman 1994) that require temporal or spatial variation in the environment (Chesson 1985). The evolved and evolving trade-offs of species life-history traits may set distinct constraints on community assembly. Thus, it is necessary to illuminate the role of particular functional traits and of trait variation within and between species for the assembly of plant communities and the functioning of ecosystems.

Knowledge about the mechanism controlling trait variation within coexisting plant communities is important. Experiments with manipulated biodiversity levels can be used to clarify the role of single species within communities (Dassler et al. 2008, Thein et al. 2008) and for ecosystem functioning (Schmid et al. 2002). Most biodiversity experiments have been conducted in grasslands and aimed to quantify biodiversity effects on plant primary productivity (Balvanera et al. 2006) as an ecosystem process. However, several studies have shown that the overall positive community response to increasing plant diversity is accompanied by mixed responses of individual species (e.g. Hooper & Vitousek 1997; Tilman et al. 1997; Hector et al. 1999; van Ruijven and Berendse 2003; Marquard et al. 2009) or different genotypes of single species (Vellend and Geber 2005; Fridley et al. 2007; Silvertown et al. 2009).

Analysing the role of individual species in ecological communities is an important prerequisite for a mechanistic understanding of community assembly and dynamics. Plant species are characterized through functional characteristics, which can modify plant fitness via their effects on growth, reproduction and survival (Violle et al. 2007). Plant functional traits are measurable morphological, physiological and phenological attributes at the individual level that directly or indirectly affect overall plant fitness (Lavorel et al. 1997; Violle et al. 2007). Plant traits have been used to establish functional classifications of organisms (Lavorel et al. 1997) or to quantify the functional diversity of communities (de Bello et al. 2009). However, these functional traits are variable between and within species (Albert et al. 2010), but so far the importance of trait variation within populations and species or between specie for the assembly of community and ecosystem processes is not well

understood. Variation in species trait values have been recently investigated as responses of functional traits to gradients in plant diversity (Gubsch et al.2011, Roscher 2011) or trait trade-offs (Reich et al. 2003, Diaz et al. 2004, Wright et al. 2005). It is supposed that the increasing variety of traits in more diverse communities lead to more efficient and complete resource use than in less diverse and, hence, more similar communities (Tilman et al. 1997; Loreau 1998). According to the "optimal allocation theory" (Bloom et al. 1985) plants tend to adjust their allocation and invest a higher proportion to organs that optimize the acquisition of the most limiting resource. A general assumption in plant biology is that intraspecific trait variation is significantly smaller than interspecific trait variation (Kraft et al. 2008). However, empirical studies have shown that functional traits can vary as much within as between species (Hodge 2004; Valladares and Niinemets 2008; Albert et al. 2010; Fajardo and Piper 2010).

When analyzing interspecific trait variation it has to be taken into account the expectation that closely related species are more likely to have similar responses to the biotic and abiotic environment (Gomez et al. 2010) and share a number of traits (e.g. symbiotic N_2 fixation of legumes) than less closely related species. Therefore, it is promising to test species phylogenetic relatedness to predict which of them may coexist. This topic has only recently received increasing attention (Webb et al. 2002; Cadotte et al. 2008; Cavender-Bares et al. 2009), since molecular sequence data of numerous species become available and phylogenetic trees can be built and integrated in analyses. In natural communities the phylogenetic pattern in species composition is often a phylogenetically non-random sample of a wider species pool (Webb et al. 2002; Cavender-Bares et al. 2009; Vamosi et al. 2009). If congeneric species are overrepresented in communities, it may be assumed that they must share ecological traits that influence community assembly and that these traits evolve more slowly than the rate of appearance of new species. Recent studies of the phylogenetic distribution of ecological traits tended to emphasize the conservative nature of plant trait evolution and their potential role for community assembly (Webb 2000; Prinzing et al. 2001; Webb et al. 2002; Ackerly 2003, 2004). However, other studies have shown that traits that influence community structure as soil moisture tolerance are evolutionary labile (Cavender-Bares et al. 2004; Silvertown et al. 1999). Thus, differences in trait values are the result of both phylogenetic inertia and natural selection of the environment (Felsenstein 1985). Strong selection pressures and fast (co-)evolution are common in nature (Thompson 2005), placing evolutionary and ecological dynamics on similar timescales (Fussmann et al. 2007). Thus, functional trait values cannot be considered as fixed characteristics of species, and it is required to consider effects of trait

evolution (local adaptation). The observed local variability would be due to the presence of several genotypes within populations. Genetic diversity can have a stabilizing effect (*sensu* Chesson 2000) on coexistence (Taylor and Aarssen 1990; Laird and Schamp 2006) of different species. Indeed, there exist a few empirical examples of large fine-scaled genetic diversity and its relationship to community-level processes (Turkington 1979; Aarssen and Turkington 1985; Vavrek 1998). For example, it has been shown that *Daphnia* species with different genotypes in the same population often display greater levels of niche differentiation than apparent among many species (Leibold and Tessier 1991; Tessier and Leibold 1997). Competitive interactions may be assumed to be a consequence and a potential cause of local genetic diversity (Vellend and Geber 2005) Therefore, local genetic and species diversity should be correlated (Vellend 2003).

While trait variation of plant species may have genetic base (Hodge 2004; Valladares and Niinemets 2008) it may also be founded on phenotypic plasticity, which are both integrated by measures of phenotypic variability (Byars et al. 2007). Phenotypic plasticity is the observed local variability of an individual and is dependent on the plasticity of given genotypes. It is well known that the ability of plant species to adapt their phenotype in response to the abiotic or biotic environment is important for optimized resource acquisition. The adaptive value of phenotypic plasticity for individual plant species is largely accepted (Sultan 1995, 2000; Schlichting & Pigliucci 1998). However, the role of phenotypic plasticity for the outcome of species interactions and its consequences for plant community assembly is not yet well understood (Callaway et al. 2003; Valladares et al. 2006). Many functional traits which are supposed to be important for the outcome of competition and associated processes of community assembly are known to be highly plastic. For instance, root length and root demography strongly respond to supply levels of nutrients (Hodge 2004), and leaf architecture and leaf number are known to be highly dependent on light availability (Pigliucci et al. 2003; Valladares and Niinemets 2008). Trait values adapted to a local environment determine a species ecological strategy (McGill et al. 2006; Westoby and Wright 2006). Environmental filters will locally limit the range of ecological strategies and may result in trait convergence of species within a community (McArthur and Levins 1967; Grime 2006; Funk et al.2008). However, phenotypic plasticity allows organisms to adjust to a large range of conditions without evolutionary change (Grassein et al. 2010). As a consequence, trait convergence within a habitat is not solely due to genetic similarities between species but may often be explained as a significant part by plastic responses, i.e. phenotypic similarity despite genetic divergence. Trait plasticity may allow species to shift their ecological strategy to fit current

environmental conditions and enable them to pass the ecological filter. Likewise, trait divergence among communities is often thought to be a consequence of community assembly rules. However, it may equally well be due to phenotypic plasticity induced by divergent environmental conditions.

For a long time it was assumed that life history traits of interacting species are uniform within species and remain unchanged over time (Agrawal et al. 2007). It was suspected that the relatively slow time scale of evolutionary changes renders an evolutionary perspective of community ecology unnecessary. However, it is unclear how much of observed variation in plant community composition is explainable by phenotypic plasticity of traits across a wide range of environments, by the phylogenetic relatedness of traits of single species within a plant community or the influence of genotypes within single species on plant community composition and functioning.

Thesis outline

The central topics of this thesis are the mechanism underlying variation in the performance of single plant species in experimental grasslands of different diversity. The thesis is based on data recorded in the Jena Experiment, a large-scale biodiversity experiment in Germany, which was designed to clarify the role of plant diversity for ecosystem functioning, element cycling and trophic interactions (Roscher et al. 2004).

In **chapter 1**, I have surveyed the role of plant diversity (species number, legume presence) on the variation of functional traits of 27 non-legume forb species and analysed their relationships to species phyloge10netic history and growth forms.

In **chapter 2**, I examined whether variation in plant individual performance in response to neighborhood diversity is due to phenotypic plasticity or to genotypic variation within single plant species. I established the offspring of seed families of five forb species collected in monocultures or in 60-species mixture after five years of selection in the Jena Experiment. Then, I transplanted or replanted the offspring to the plot of their own origin and into the respective monoculture or 60-species mixture to assess whether five yerars of selection in plant communities of different diversity led to a genetic differentiation or whether trait variation is exclusively due to phenotypic plasticity of the investigated forb species.

In **chapter 3**, I explored whether plants of *T. officinale* originating from sites with presumably different selection regimes, either a density-independent mortality by weeding (r-selection regime) or density- dependent mortality by increasing interspecific competition along a

species richness gradient (K-selection regime) over 5 years have developed traits expected under r- or K-selection in a common environment.

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Chapter 1

Functional traits and trade-offs of forb species in response to increasing plant diversity

Annett Lipowsky, Christiane Roscher, Jens Schumacher, Stefan Michalski, Marlén Gubsch, Nina Buchmann, Ernst-Detlef Schulze & Bernhard Schmid (Manuscript)

Abstract

1. Plant functional traits are increasingly used to identify plant strategies and their relation to community dynamics and ecosystem functioning. However, trade-offs between different plant functions, trait variation in response to changes in the abiotic and biotic environment and its limitation through evolutionary relationships among co-existing species have been rarely tested empirically.

2. Here, we studied twelve functional traits associated with light and nutrient acquisition and reproduction and its variation in response to increasing plant diversity in 27 non-legume forb species with different growth forms and phylogenetic history in a large grassland biodiversity experiment (Jena Experiment).

3. Variation in traits associated with light acquisition (i.e. shoot height, leaf length, specific leaf area, foliar $\delta^{13}C$ values) indicated increasing efforts for light acquisition at increasing species richness and when legumes were present, but trait variation differed among forbs with different growth forms. Effects of legume presence on an improved nitrogen nutrition of forbs reflected in higher leaf nitrogen and shoot concentrations differed among forb species irrespective of growth form. Functional trait values and variation in response to increasing plant diversity were phylogenetically conserved to some degree, particularly for physiological traits associated with nitrogen acquisition. Nevertheless, species-specific differences in the magnitude of trait variation in response to increasing plant diversity and highly variable correlation structures among traits provided clear evidence for species uniqueness in plant diversity–trait variation relationships.

4. The results of our study emphasize that current environmental variation in plant communities of increasing diversity surpass effects of shared ancestry on functional trait expression of forb species. These plastic responses are the prerequisite for niche partitioning among them and their coexistence in multi-species assemblages.

Keywords: biodiversity, functional traits, phylogeny, species identity, trait variation

Introduction

Concerns about the consequences of currently unprecedented rates of species loss have stimulated the implementation of numerous experimental studies on biodiversity–ecosystem functioning relationships. These experiments have verified the important role of plant diversity, i.e. the number, identity and composition of plant species present in a community, for ecosystem processes such as primary productivity (e.g. Balvanera *et al*. 2006; Díaz *et al.* 2006). At increasing plant diversity, intra-specific interactions among plants are likely to be replaced by inter-specific interactions and this may lead to a greater proportional light, water and nutrient availability if species are complementary in their resource use (Naeem *et al.* 1994; Tilman *et al.* 1997), but the biological mechanisms of niche partitioning are not well understood so far.

The biotic and abiotic environment which plant individuals experience in communities of increasing plant diversity varies in multiple ways due to different plant neighbor identities and densities (Marquard *et al.* 2009), a more complete use of resources such as nutrients (Palmborg *et al.* 2005; Oelmann *et al.* 2007), light and space (Spehn *et al.* 2000; Lorentzen *et al.* 2008) and changing biotic interactions with higher trophic levels such as herbivores and pathogens (Knops *et al.* 1999). Plants may adjust to environmental changes through plastic responses at different levels of plant organization, encompassing growth and morphology at the organ- or whole-shoot level, physiology, phenology and reproduction, which consequently affects survival and plant fitness. Plant functional traits have been suggested as useful tools to identify plant strategies in resource acquisition and use, to reflect species adjustment to the local environment and to understand mechanisms of plant community assembly and stability (Diaz & Cabido 2001; Violle *et al.* 2007). For instance, typical morphological adjustments in response to reduced light supply in the canopy shade of taller neighbors are the formation of larger and thinner leaves (shade tolerance) or an increase in stem length (shade avoidance) (Callaway *et al.* 2003; Valladares & Niinemets 2008). stable C isotope ratios ($\delta^{13}C$) in plant tissue give valuable information about photosynthetic activity or stomatal conductance, which depend on light availability, air humidity and plant nutrition (Farquhar *et al.* 1989; Dawson *et al.* 2002). Stable N isotope ratios ($\delta^{15}N$) in non-legume plant species may provide information about the provision of depleted legume-derived N in communities with legumes or a shift in the uptake of different N forms (Jumpponen *et al.* 2002; Craine *et al.* 2009; Gubsch *et al.* 2011).

Plants are able and very flexible to shift allocation towards organs that optimize the acquisition of the most limiting resource (Bloom *et al.* 1985; Poorter & Nagel 2000). The

complex environmental changes at increasing plant diversity require a coordinated response to solve a trade-off in balancing different functions, which may lead to the differentiation of species-specific ecological strategies and consequently promote complementarity among co-occurring species. However, the differentiation of ecological strategies among co-existing species might be limited through correlated evolutionary divergence of traits due to ancestral similarities and the persistence of trait combinations within lineages of phylogenetic branches (Felsenstein 1985; Silvertown & Dodd 1995). Plant communities are likely to force reciprocal evolutionary influences on resource use and interactions if species associations develop over longer evolutionary time-scales, but plant functional traits differ widely in their evolvability. Rapid evolutionary dynamics and stasis are assumed to co-occur within and among taxa (Thompson 2009). Therefore, a mechanistic understanding of niche differentiation and species adjustment to environmental changes in plant communities of increasing diversity requires considering the relationships between phylogenetic relatedness and ecological similarity.

So far, functional trait variation and trade-offs (negative correlations) as well as their dependency on the phylogenetic relatedness of species have not been in the focus of biodiversity–ecosystem functioning research, but are promising in providing an integrated view of diversity-related changes in plant functioning. In the current study, we investigated functional traits of aboveground plant organs related to carbon and nitrogen acquisition and use, and measured plant performance in terms of biomass and reproductive structures in experimental communities of increasing species richness and with presence or absence of legumes. The study was conducted in a long-term biodiversity experiment (Jena Experiment; Roscher *et al.* 2004) on 27 herbaceous species of varying growth forms representing a broad range of plant families which differ in their phylogenetic relatedness. We specifically focused on non-leguminous forb species which provide a broad spectrum of phylogenetic relationships to assess whether a differential expression of their functional traits and their variation in response to increasing plant diversity may allow for complementary resource acquisition and use among them. Specifically, we ask the following questions:

(1) Are there common plastic responses (direction of trait variation) of all forbs species to increasing species richness or to the presence of legumes in the experimental plant communities and does this variation depend on the phylogenetic history of the investigated species?

(2) Are traits associated with light or nutrient acquisition and use and reproduction of different forbs species phylogenetically conserved?

(3) Are there species-specific differences in functional trait variation of forb species in response to increasing plant diversity and may these differences be related to different growth-forms?

Material and methods

DESIGN OF THE JENA EXPERIMENT

This study was carried out in a large grassland biodiversity experiment, the Jena Experiment, established in spring 2002 (Roscher *et al.* 2004). The experimental site is a former arable field located in the floodplain of the river Saale near Jena (50°55` N, 11°35` E, 130 m a.s.l; Germany). The area around Jena is characterized by mean annual air temperatures of 9.3°C and a mean annual precipitation of 587 mm (Kluge & Müller-Westermeier 2000). The soil of the experimental area is developed from up to 2 m thick fluvial sediments and varies in texture from sandy loam near the riverside to silty clay with increasing distance from the river. Because of this gradient in soil characteristics, the field site was divided into four blocks parallel to the river.

Eighty-two experimental plant communities of varying species richness and composition were assembled from a pool of 60 species typically found in Central European semi-natural, species-rich grasslands (*Molinio-Arrhenatheretea*, Ellenberg 1988). Based on a cluster analysis of a literature-based matrix of morphological, physiological and phenological traits the 60 plant species were classified into four functional groups: 16 grasses, 12 small forbs, 20 tall forbs and 12 legumes (Roscher *et al.* 2004). The five species-richness levels of 1, 2, 4, 8 or 16 species were near-orthogonally crossed with four levels of functional group richness (1 to 4). Each species-richness level had 16 replicates (except for the 16-species mixtures where pure legume and small-forb communities were not possible). In addition, four plots were established with all 60 species. Mixture compositions were determined by random draws from the species pool and communities of the same richness level were allowed to partly overlap with regard to the species they contained. Each community was sown on a plot of 20 x 20 m. Monocultures of all experimental species were established on smaller plots of 3.5 x 3.5 m. Total sowing density was 1000 viable seeds per square meter. In mixtures, all species were sown with equal proportions, i.e. sowing densities per species were 1000 divided by species richness of the plot, yielding a substitutive experimental design. Plots were weeded twice per year (early April and July) to maintain target species compositions. Each year, plots where

mown twice at the end of May and August to mimic the usual management of extensive hay meadows. The plots did not receive any fertilizer.

DATA COLLECTION

In this study, traits of 27 forb species of the experimental pool of 60 species (Table 1) were analyzed. Measurements took place during two harvest campaigns at estimated peak biomass before mowing (May and August 2006) in monoculture and mixture plots. For the 60-species mixtures two plots were sampled. Plant modules (= individual shoots) served as basic unit for all measurements because in many herbaceous species a reliable distinction of plant individuals is not possible. Modules are defined as plant parts that would be able to grow independently if separated from the genet, the plant individual derived from a sexually produced seed (Harper 1977). Five modules per species and plot were studied, when only vegetative plants occurred. Three vegetative and three reproductive modules were studied in cases where modules of different life stages were available. Transects divided into segments at 50 cm distance in large and 25 cm distance in small plots where established perpendicular to the plot margin (excluding the outer 70 cm) and modules nearest to each segment were chosen for sampling.

In the field, the height of the chosen module and canopy height of the immediately surrounding vegetation were measured. Thereafter, modules were cut off at ground level and put in sealed plastic bags into a cool box to prevent dehydration until further processing in the laboratory. There, the maximum shoot length (stretched module length), the length of the three longest leaves and of 3–5 central internodes of the main shoot axis were recorded. The stem diameter was measured in the middle of the main axis with a caliper. Afterwards, modules were separated into compartments (stems and higher-order axes = supporting tissue, leaves, reproductive parts = inflorescences and fruits). The area of 3–10 (depending on leaf size) fully developed leaves including petioles from the upper part of the plant module was determined with a Leaf Area Meter (LI-3000A Portable Area Meter, Li-COR, Lincoln, USA). Afterwards, all plant material was dried for 48 h at 70°C and weighed. For subsequent chemical analyses, samples of measured leaves and all residual compartments, respectively, were pooled per plot and species (separately for vegetative and reproductive modules if available) and ground with a ball mill. Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios and elemental concentrations were analyzed from measured leaf material (3 mg and 0.8 mg, respectively), with an isotope-ratio mass spectrometer (Delta C prototype IRMS, Finnigan MAT). Sample ratios of $^{15}N/^{14}N$ are expressed relative to the international standard for

atmospheric N_2, and sample ratios of $^{13}C/^{12}C$ refer to the VPDB standard for C. Values are given in per-mil relative to the standards. The remaining bulk plant material was analyzed for nitrogen concentrations with an elemental analyzer (Vario EL Element Analyzer, Elementar, Hanau, Germany) (Table 2).

DATA ANALYSES

Trait values were averaged per species, harvest time, life stage (vegetative and reproductive, respectively) and plot. Growth forms were classified into reptant, rosulate and semirosulate (see Table 1). Reptant species have a creeping growth form with aboveground runners, in rosulate species leaves are exclusively at the base, while semirosulate species form stems with leaves when they develop inflorescences. Because most studied herbaceous species reached the flowering stage either in early summer (before first mowing) or in late summer (before second mowing) differences between harvests could be analyzed for vegetative modules only, whereas reproductive modules were often only available once. Here, we chose for each species the date where its flowering phenology was most advanced.

Since individual species were randomly assigned to mixtures, i.e. experimental plots, the design has a certain degree of unbalancedness with varying total number of occurrences per species and traits of several species measured on the same plot. We therefore considered species effects and plot effects as independent crossed random effects and analyzed the influence of experimental factors and species growth form on species traits using linear mixed-effects models of the statistical software system R 2.11.1 (R Development Core Team 2010, http://www.R-project.org; package *lme4*; Bates & Sakar 2006). Fixed effects used in our models where growth form (reptant vs. rosulate vs. semirosulate; GF), species richness (as log-linear term; SR), presence/absence of legumes (Leg), interaction terms between growth form and species richness (GF x SR) as well as harvest time for the vegetative modules (see appendix). Nevertheless, the random effects associated with different species cannot be considered stochastically independent, because species have different degrees of phylogenetic similarity. To account for this phylogenetic dependence we constructed a phylogeny (Table 1, Fig. 1) for the 27 forb species based on published *rbc*L gene sequence data obtained from the GenBank database. Because of missing data, sequence information of closely related species was used for 10 out of 27 species (see Appendix Table 5). After alignment, a maximum likelihood tree was computed using the default settings implemented in the program *dnaml* of the software *PHYLIP* version 3.6 (Felsenstein 2004). The resulting correlation matrix was used to describe the correlated response of species in our experiment. Because we were not

able to describe our particular dependence structure using available standard packages for linear mixed models, we derived maximum-likelihood estimates for model parameters by directly maximizing the likelihood function using the general purpose optimization function *constrOptim* of the statistical software system R (see appendix for the R code).

Likelihood-ratio tests were applied to compare the models obtained by step-wise addition of terms thereby assessing the significance of the fixed effects for both computations (with and without inclusion of the phylogenetic correlation matrix). For the computation without any phylogenetic contrast the model with the lowest AIC (Burnham & Anderson 2002) for fixed effects was chosen and the maximum set of random effects was simplified stepwise starting with the species identity within richness term (SpI[SR]) followed by species identity term (SpI) applying the maximum likelihood method and likelihood ratio tests. In the computations with phylogenetic contrasts for the random species effect we considered two alternatives, a random intercept model accounting for general species differences only and a random intercept and slope model, additionally accounting for species-specific response to species richness.

Additionally, we tested for phylogenetic signal in single traits by calculating Blomberg's K statistic (Blomberg *et al.* 2003) using code from the R package *picante* (Kembel *et al.* 2010). This statistic quantifies trait variation according to a given phylogeny variance-covariance matrix, which is compared against trait values expected under Brownian motion assumption. Based on 1000 random shuffles of the tips of the tree the significance of the K-values was assessed and 95% confidence intervals were calculated (see Cadotte *et al*. 2009 for R script). Blomberg`s K statistic is bounded between 0 and 1, where values near 0 suggest a lack of a phylogenetic signal, while a value of K close to 1 indicates that close relatives are very similar (i.e. Browian character evolutions). K values greater than predicted by the null distribution based on the randomization procedure indicate that close relatives are more likely to share common characteristics than expected by chance.

To test for trait correlations across species, we calculated Pearson correlation coefficients for all pairwise combinations of mean trait values (averaged across all plots) separately for vegetative and reproductive plant modules. To assess the dependency of trait correlations on plant diversity in terms of species richness or legume presence/absence, we used trait data corrected for block effects to derive correlation matrices of regression slopes against species richness and legume presence (residual slopes after accounting for species richness effects). Pearson correlation coefficients were calculated after correcting for shared evolutionary histories (phylogenetic independent contrasts, PICs) using the package *ape* (Paradis *et al.*

2004) of the statistical software R. PICs account for covariance in trait values for closely related taxa by estimating differences in trait values between lineages and standardizing these differences by the estimated time of divergence.

In all analyses, data were log- or arcsine-square root (ratios) transformed if necessary to meet the assumptions of normality and homogeneity of variances.

Results

All measured traits differed significantly among the investigated forb species, irrespective of their developmental stage as vegetative (available for 27 species) or reproductive (available for 25 species) modules (Table 3, 4). Traits measured in vegetative modules differed significantly between early and late summer (Table 3) except for $\delta^{15}N$ values and module mass. In the following, we firstly show results based on statistical modeling without accounting for phylogenetic relatedness among taxa, and secondly we compare these results to models which included the phylogenetic relationships among the studied species.

TRAITS ASSOCIATED WITH LIGHT ACQUISITION

All traits associated with light acquisition (i.e. module height, leaf length, SLA, foliar $\delta^{13}C$ values; see Table 2) varied among different growth forms of forb species (with exception of leaf length of reproductive modules). Increasing species richness and legume presence had significant effects on the expression of these traits (with exception of SLA of reproductive modules) irrespective of module life stage (Table 3, Fig. 2a–d). Reptant and rosulate species had the lowest height and leaves with larger SLA and lower $\delta^{13}C$ values than forb species with semirosulate growth. Significant interactions between growth form and species richness (GF x SR) for light acquisition traits of reproductive modules with exception of plant height suggested differential responses to increasing species richness among forb species dependent on their growth form (Table 3). On average, rosulate species had the longest leaves which the most pronounced elongation with increasing species richness (Fig. 2b). Tall-growing semirosulate species showed the lowest increase in SLA in response to increasing species richness compared to rosulate and reptant species (Fig. 2c). Reptant species experienced the largest decline in their foliar $\delta^{13}C$ values with increasing species richness (Fig. 2d).

TRAITS ASSOCIATED WITH NITROGEN ACQUISITION AND USE

Traits associated with nitrogen acquisition and use (i.e. mass-based leaf nitrogen, module biomass:N ratio, foliar $\delta^{15}N$ values; see Table 2) did not differ among forb species with different growth forms, with exception of biomass:N ratios in vegetative modules (Table 3, Fig. 2e–g). Foliar $\delta^{15}N$ values generally decreased with increasing species richness, but species-richness effects depended on species identity (Table 3, Fig. 2e). Biomass:N ratios and leaf nitrogen concentrations were higher when forb species grew with legumes and differed between early and late summer. Seasonal differences in foliar $\delta^{15}N$ values did not occur.

MODULE MASS

Module mass varied among species with different growth forms. Vegetative and reproductive modules of semirosulate forb species had the highest biomass, while modules of reptant forbs had the lowest biomass (Fig. 2h). Increasing species richness and legume presence had positive effects on biomass of reproductive modules (Table 3), while positive species-richness effects on biomass of vegetative modules depended on species identity were found.

TRAITS ASSOCIATED WITH REPRODUCTIVE STRUCTURES

Rosulate and semirosulate plants form stems only when they develop inflorescences. Mean internode length, stem diameter and stem:leaf ratio differed significantly among species with different growth forms (Table 4, Fig. 2i–k). Semirosulate forb species produced the longest internodes, thickest stems and had the largest stem:leaf ratios. Legume presence initiated the formation of longer internodes, thicker stems and larger stem:leaf ratios. Increasing species richness did not affect the measured reproductive traits (Table 4). The inflorescence mass fraction was not related to the experimental factors or growth form, but depended on species identity (Table 4, Fig. 2l).

MODEL CALCULATION ACCOUNTING FOR PHYLOGENETIC RELATEDNESS

When accounting for phylogenetic relatedness among forb species the likelihood for significant effects of the experimental factors and growth form declined (Table 3, 4). Species-richness effects on traits associated with light acquisition did not remain statistically significant at all (e.g. mean leaf length) or the likelihood ratios of models calculated with the phylogenetic correlation matrix were considerably lower (Table 3). The inclusion of phylogenetic relatedness caused only small differences in the predictive value of growth form (except for SLA of reproductive modules), legume presence or seasonal differences between early and late summer in light-acquisition traits. Differential effects of increasing species

richness on mean leaf length of forb species increased after accounting for phylogeny (Table 3).

After accounting for phylogeny, we found stronger differences in mass-based leaf nitrogen and foliar $\delta^{15}N$ values of vegetative modules among forb species with different growth forms. The inclusion of phylogenetic relatedness did not influence effects of other model terms on traits associated with nitrogen acquisition, with exception of species-richness effects on foliar $\delta^{15}N$ values of vegetative modules (Table 3).

While growth form had no effects on variation in inflorescence mass fraction (IMF) without accounting for species` phylogenetic relatedness, differences in IMF among forb species with different growth forms and in response to increasing species richness became statistically significant after accounting for phylogenetic relationships (Table 4). In contrast, after correcting for phylogeny the dependence of internode length on growth form disappeared. In addition, species-dependent species-richness effects on the variation of stem:leaf ratios did not remain statistically significant (Table 3). Species-richness effects on module biomass also became non-significant after accounting for phylogeny.

PHYLOGENETIC SIGNAL

Among the 8 traits studied in both vegetative and reproductive modules, there was a significant phylogenetic signal for mean values of foliar $\delta^{13}C$ values (vegetative modules: $K = 0.339$, $K_{rand} = 0.146$, 95% CI = 0.056–0.310; reproductive modules: $K = 0.396$, $K_{rand} = 0.168$, 95% CI = 0.062–0.344). We also detected a phylogenetic signal in biomass:N ratios ($K = 0.402$, $K_{rand} = 0.149$, 95% CI = 0.057–0.33) and mass-based leaf nitrogen ($K = 0.374$, $K_{rand} = 0.153$, 95% CI = 0.060–0.325) of vegetative modules. Variation of mass-based leaf nitrogen of reproductive modules in response to increasing species richness ($K = 0.378$, $K_{rand} = 0.161$, 95% CI = 0.055–0.361) was more similar among phylogenetically more closely related forb species than expected by chance. The response of forb species to legume presence was phylogenetically more clustered in biomass:N ratios of vegetative modules ($K = 0.346$, $K_{rand} = 0.148$, 95% CI = 0.058–0.328) and leaf nitrogen concentrations (vegetative modules: $K = 0.438$, $K_{rand} = 0.15$, 95% CI = 0.058–0.346; reproductive modules: $K = 0.944$, $K_{rand} = 0.177$, 95% CI = 0.066–0.350) than expected by chance. Additionally, there was a detectable phylogenetic signal in response to legume presence for foliar $\delta^{13}C$ values of reproductive modules ($K = 0.657$, $K_{rand} = 0.179$, 95% CI = 0.063–0.375). There was no significant phylogenetic signal in other traits, neither in their mean values nor in response to species richness or legume presence.

TRAIT CORRELATIONS

VEGETATIVE MODULES

Traits which are directly associated with light acquisition as module height, leaf length and foliar $\delta^{13}C$ values were positively correlated with module biomass except for SLA. Light-acquisition traits had single positive correlations to nitrogen-acquisition traits, e.g. module height with biomass:N ratios, maximum leaf length with biomass:N ratios and mass-based leaf nitrogen, and foliar $\delta^{15}N$ values with foliar $\delta^{13}C$ values. SLA correlated negatively with maximum leaf length (Fig. 3a).

Species-richness effects on variation in module biomass correlated positively with variation in leaf length, foliar $\delta^{13}C$ and foliar $\delta^{15}N$ values and negatively with variation in SLA. Species variation in module height in response to increasing species richness was positively correlated with all traits characterizing nitrogen acquisition and use, i.e. mass-based leaf nitrogen, biomass:N ratios and foliar $\delta^{15}N$ values. Declining SLA in response to species richness correlated with decreasing foliar $\delta^{13}C$ and foliar $\delta^{15}N$ values in response to increasing species richness (negative correlations; Fig. 3c).

Variation in module height and partly variation in leaf length in response to legume presence or absence correlated positively with variation in module mass, biomass:N ratios and mass-based leaf nitrogen (Fig. 3e). Consequently, a better N supply when legumes were present correlated with increasing efforts for light acquisition. Species variation in SLA in response to legume presence correlated negatively with variation in foliar $\delta^{13}C$ values and positively with variation in biomass:N ratios and mass-based leaf nitrogen. Legume effects on variation in module mass correlated positively with variation in foliar $\delta^{13}C$, biomass:N ratios, mass-based leaf nitrogen and foliar $\delta^{15}N$ values (Fig. 3e).

REPRODUCTIVE MODULES

The number of significant correlations increased from 10 in vegetative modules to 30 in reproductive modules (14 correlations between vegetative traits, and 16 correlations between vegetative and reproductive traits and within reproductive traits). Species mean values of traits associated with module size, light acquisition and reproduction had mostly significant correlations. While most trait correlations were positive, SLA correlated negatively with growth height and maximum leaf length, and stem:leaf ratios correlated negatively with foliar $\delta^{13}C$ and foliar $\delta^{15}N$ values (Fig. 3b).

Variation in height in response to increasing species richness correlated positively with variation in reproductive traits and module biomass and negatively with variation in module biomass:N ratios and foliar $\delta^{15}N$ values. Variation in biomass:N ratios in response to increasing species richness correlated negatively with variation in inflorescence mass fraction (IMF) and stem:leaf ratio (Fig. 3d).

Variation in height in response to legume presence correlated negatively with stem diameter and inflorescence mass fraction, but was positively related to variation in stem:leaf ratio. In contrast to variation in response to species richness, variation in stem diameter in response to legume presence was positively correlated with variation in SLA and negatively correlated with variation in module biomass, growth height, mean internode length and stem:leaf ratio (Fig. 3f). Similar to the response to increasing species richness significant correlations between mean internode length and biomass:N ratios as well as $\delta^{15}N$ values were found dependent on legume presence. Variation in SLA, module biomass, biomass:N ratios and stem diameter in response to legume presence had the highest number of significant correlations among all investigated traits.

Discussion

Based on 27 forb species common in Central European mesophilic grasslands we used experimental plant communities of varying species richness and composition to explore the issue whether the phylogenetic relatedness between these forb species reflects their ecological similarity and thus influences species interactions and processes governing community assembly (Wiens & Graham 2005). The restriction of our analyses to forb species was guided by their unique variety in growth forms and phylogenetic lineages compared to other commonly used functional groups of grassland species such as legumes or grasses. Thus, we focused on (1) whether morphological and physiological traits associated with light and nitrogen acquisition and use and performance in terms of biomass and reproductive structures are related to their growth forms or to which degree they depend on phylogenetic constraints. Particularly we aimed to investigate (2) whether different forb species have developed a variety of strategies to adjust to complex environmental changes such as variation in plant diversity allowing for niche segregation and complementary resource use.

TRAITS ASSOCIATED WITH LIGHT ACQUISITION

Plant communities are characterized by a light climate which is strongly predictable in a vertical direction, i.e. light quantity and quality change over the canopy profile dependent on

stand density and structure (Jones 1992). All investigated forb species expressed some adjustment in morphological traits associated with light acquisition at increasing plant diversity, but these plastic responses resulted in divergent trait values dependent on species identity and growth forms (Fig. 2 a–d). Semirosulate, mostly tall-growing species, which forage vertically for light, increased shoot length. In contrast, reptant and rosulate species, which are not able to grow out of the vegetation shade because of inherent growth restrictions due to mechanical constraints (Niklas & O`Rourke 1982), increased their leaf area per unit leaf biomass (SLA) which enables these small-statured plants to optimize light capture and photosynthetic carbon gain (Poorter *et al.* 2006; Roscher *et al.* 2011a).

Variation in SLA in response to increasing plant diversity was dependent on life stage. Vegetative modules of all growth forms, which mostly consist of a leaf rosette, had higher SLA at higher levels of plant species richness. In contrast, variation in SLA of reproductive modules in response to increasing species richness was less pronounced. Resource availability and plant density are critical determinants for inflorescence development of herbaceous species (Sugiyama & Bazzaz 1998). Most forb species must reach a critical plant size for flower initiation (Lovett Doust & Lovett Doust 1988), thus taller reproductive plants obviously do not invest further in height compensation through increasing SLA. Niinemets (2004) demonstrated significant changes in species plasticity with age in *Leontodon hispidus*, thus showing that even for a single species there is a broad spectrum of morphological responses to light availability.

Plant stable C isotope ratios ($\delta^{13}C$) are an integrative long-term measure related to stomatal conductance and photosynthetic activity which are dependent on light, water and nutrient availability (Farquhar *et al.* 1989). Foliar $\delta^{13}C$ value on average decreased with increasing species richness and when legumes were present. In contrast, foliar N concentrations did not vary in response to increasing species richness, but some forb species had higher leaf N concentrations in communities with legumes. Carbon assimilation rates of leaves are a linear function of their nitrogen concentrations (Evans 1989). Thus, a better nitrogen nutrition may decrease discrimination against $\delta^{13}C$ (Toft *et al.* 1989), resulting in less negative bulk $\delta^{13}C$ values. In our study, some tall-growing species had less negative $\delta^{13}C$ values with increasing species richness, suggesting that N availability was the strongest control for foliar $\delta^{13}C$ which is in accordance with results by Jumpponen *et al.* (2005). In contrast, a typical response of small-statured species growing at reduced light supply deep in the canopy are lower rates of photosynthetic rates (Long *et al.* 1994; Roscher *et al.* 2011a). Jumpponen *et al.* (2005) found a negative correlation between $\delta^{13}C$ and light availability in smaller plants at increasing plant

diversity due to canopy shading by dominant, larger species. However, we cannot exclude diversity-dependent variation in the isotopic composition of the source CO_2 in lower canopy layers of denser plant stands due to low air turbulence, and increased soil and plant respiration resulting in a remarkable CO_2 enrichment near to the ground at high standing biomass (Roscher *et al.* 2011a) and presumably in lower $^{13}C/^{12}C$ ratios (Buchmann *et al.* 2002).

TRAITS ASSOCIATED WITH NITROGEN ACQUISITION AND USE

Symbiotically N_2-fixing legumes may improve soil nitrogen supply for neighboring non-legume plant species through rhizodeposition or decomposition of their litter with a low C:N ratio (Hartwig 1998). Forb species in our experimental species pool differ largely in rooting depths and types (Roscher *et al.* 2004) and may vary in their ability to exploit high-nutrient patches, their relative uptake rates of different N forms and temporal patterns of nutrient uptake (Jumpponen *et al.* 2002). Forb species such as *G. pratense* and *R. acetosa* with belowground storage organs had higher leaf N concentrations than other forbs. Nitrogen accumulation in storage organs in times of high N supply in the soil and subsequent reallocation of such stored N to aboveground plant tissue has been observed in several studies (Volenec *et al.* 1996; Louahlia *et al.* 1999). The natural abundance of foliar $\delta^{15}N$ is highly variable among plants in natural ecosystems (Craine *et al.* 2009) due to the assimilation of different N forms (e.g. nitrate, ammonium), association with different types of mycorrhizal fungi and plant internal fractionation.

It is well known that $\delta^{15}N$ values vary between different plant families and in response to resource availability (Högberg 1997; Craine *et al.* 2009). In our study, forb growth form had little effects on foliar $\delta^{15}N$, but species identity was highly significant in analyses of vegetative modules. Within-plant recycling of nitrogen, fractionation between plant organs or different rooting depth (Evans 2001) could explain different $\delta^{15}N$ values among different plant families in vegetative modules. Contrary to our expectations, the presence of legumes did not affect foliar $\delta^{15}N$ signature of forb species although a large proportion of legume N was derived from symbiotic N_2 fixation in our experiment (Roscher *et al.* 2011b). However, the better nitrogen nutrition in communities with legumes suggests that legumes indirectly facilitated neighboring forb species through soil nitrogen sparing (Temperton *et al.* 2007). However, a significant species richness effect showed that other mechanisms affect variation in foliar $\delta^{15}N$ in our study. These differences in foliar $\delta^{15}N$ values may be due to different N sources ($NO3^{-}$, NH4+) and differences in N mineralization rates (Kahmen *et al.* 2008).

PHYLOGENETIC RELATEDNESS

In our study, the average relatedness among different families and genera and their representation in the experimental species pool was very heterogeneous. If functional differences among species are related to the time of their phylogenetic divergence, related species are likely to be ecologically more similar (niche conservatism), i.e. sharing similar functional traits and corresponding in their response to the biotic and abiotic environment (Felsenstein 1985; Harvey & Pagel 1991; Gómez *et al.* 2010). Although Silvertown *et al.* (2001, 2006) found no phylogenetic signal in community assembly of meadow vegetation, niche conservatism and slow evolution of plant traits was supported by other studies (Webb *et al.* 2002)

All plant traits associated with light-acquisition were significantly affected by increasing species richness without accounting for their phylogenetic relatedness. However, when we accounted for phylogenetic correlations among forb species, the species richness effect did not remain statistically significant (Table 3). Thus, phylogenetically more closely related species responded more similarly in their light-acquisition traits to increasing species richness than expected by chance and are likely to express more similar strategies when competing for light. In contrast, we found little evidence for effects of phylogenetic relatedness in traits associated with nitrogen acquisition in response to increasing species richness or legume presence. This suggests trait convergence, i.e. that strategies related to nutrient acquisition and use in multi-species assemblages evolved in parallel in distant lineages or underwent little changes since species evolved from common ancestors. Many studies have shown that plant nutrient concentrations are influenced by nutrient availability as well as species-specific differences in growth form, physiology and life history (Aerts & Chapin 2000), which is in line with our results showing significant effect of growth form in traits associated with nitrogen acquisition and use in vegetative modules after accounting for phylogenetic relatedness.

PHYLOGENETIC SIGNAL

Furthermore, variation in plant nutrient content may partly be attributable to species taxonomic relationships (Thompson *et al.* 1997) or phylogenetic affiliation (Broadley *et al.* 2004). The results of the Blomberg`s K statistics for leaf N concentrations and biomass:N ratios showed a significant degree of phylogenetic niche conservatism in vegetative forb modules. It is likely, that nitrogen concentration of leaves of phylogenetically more closely related forbs also affected foliar $\delta^{13}C$ values (Toft *et al.* 1989) and may explain the significant

phylogenetic signal. In contrast, the lack of phylogenetic signal in the generative modules may reflect the allocation of N from foliar tissue to reproductive organs in the flowering period. Thus, our findings are not in line with the expectations that physiological traits have lower levels of phylogenetic signal compared to other traits (Carvahlo *et al.* 2006).

Forb species were on average strongly affected by the presence of legumes indicated by significantly higher mass-based leaf N, irrespective of the phylogenetic correlation between the forb species (Table 3). However, Blomberg`s K values of legume effects on leaf N concentrations and biomass: N ratios, suggested a significant phylogenetic signal in the degree of response. More closely related forb species responded with a more similar increase in mass-based leaf nitrogen and whole shoot nitrogen. Thus, the observed patterns are likely a product of shared ancestry but depend on the diversity of growth forms evolved in different lineages of forb species, which is are closely related to different root characteristics (Jumpponen *et al.* 2002; Kahmen *et al.* 2006).

TRAIT CORRELATIONS

The number, strength and direction of trait correlations varied considerably between vegetative and reproductive modules. Correlation patterns are not easily predictable from expected relationships among functional traits, but may depend on life stage and life form. For instance, among woody plant species trait correlations differ between juvenile and mature plants (Cornelissen *et al.* 2003). In our study, all light-associated traits correlated positively with module height in vegetative modules. Although investment in height growth increases the chance for improving light acquisition, species differed in their variation in SLA. Vegetative modules of tall-growing forbs obviously reach higher canopy levels with improved light supply through height growth, whereas small-growing species increased SLA to tolerate the lower light availability. Evidence for growth limitations of small-statured forbs at low light supply was also provided from the correlogram at the reproductive stage. Despite increasing their SLA, investment into reproductive structures (inflorescence mass fraction) in small-statured forbs was reduced compared to tall-growing forbs. Many significant negative correlations among regression slopes of traits against increasing species richness or legume presence between traits associated with light and nutrient acquisition and those of characterizing reproduction emphasized that species developed specific strategies for reproduction in an environment with diverse neighborhood. The number of significant negative correlations was also higher between traits characterizing vegetative growth in reproductive modules (see Fig. 3c–f).

Conclusions

In most previous studies using plant traits to predict community and ecosystem functioning, species have been described by mean functional traits, assuming that intraspecific variability in plant traits can be neglected in comparison to interspecific variation. Only recently, intraspecific trait variation came into the focus of trait-based approaches (e.g. Albert *et al.* 2010; Jung *et al.* 2010; Hulsdorf & Swensson 2010). Here, we showed that forb species representing different growth forms and phylogenetic histories, had unique functional trait combinations which are a prerequisite for complementarity among them. Increasing plant diversity induces variation in functional traits which are known as typical plant responses to changes in light and nutrient availability—supporting the view that main ecological strategies are robust across different species (Reich *et al.* 2003). Nevertheless, species-specific differences in the magnitude of trait responses to increasing plant diversity and a highly variable correlation structure among traits provided clear evidence for species uniqueness in plant diversity–trait variation relationships. Our results that variation in light- and nitrogen-acquisition traits of forb species, differ in their phylogenetic conservatism suggest that even among congeneric species different ecological strategies have evolved (Mayfield & Levine 2010). These species-specific strategies may allow an increasing complementarity in more diverse systems. Co-occurrence with an increasing number of other plants (i.e. species richness) or neighboring species with particular traits (i.e. effects of legumes) has an influence on functional trait expression of forb species.

Acknowledgments

The Jena Experiment is funded by the German Science Foundation (FOR 456) with additional support from the Max Planck Society and the University of Jena and coordinated by W.W. Weisser. We thank all people involved in the management of the experiment, especially the gardeners, many student helpers and A. Weigelt for coordinating the field site. We acknowledge Katrin Nyakatura, Juliane Kelm, Maja Weber, Monique Seyfarth and Martina for help during the measurement campaigns and sample preparation. I. Hilke and H. Geilmann conducted chemical and stable isotope analyses.

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Table1: Number of plots where the investigated forbs were sampled in communities with or without legumes. The number of plots where the original seed mixtures contained the respective forb species is given in parentheses.

Species	Growth form	Family	Species richness									
			1	2		4		8		16		60
			without Legumes	without Legumes	with Legumes	without Legumes	with Legumes	without Legumes	with Legumes	without Legumes	with Legumes	with Legumes
Ajuga reptans L.	reptant	Lamiaceae	1	0	0	1	0	3	0	0	5(6)	1(2)
Achillea millefolium L.	semirosulate	Asteraceae	1	0	0	1	0	1	0	2	2	2
Bellis perennis L.	rosulate	Asteraceae	1	1	0	0	0	1	0	1	2	2
Carum carvi L.	semirosulate	Apiaceae	1	1	0	0	0	1	0	0	2	2
Centaurea jacea L.	semirosulate	Asteraceae	1	0	0	0	0	0	0	1	2	2
Cirsium oleraceum (L.) Scop.	semirosulate	Asteraceae	1	0	0	0	0	0	0	1	1	2
Crepis biennis L.	semirosulate	Asteraceae	1	0	0	1	1	0	1	1	2	2
Daucus carota L.	semirosulate	Apiaceae	1	2	1	0	0	0	1	1	0	2
Galium mollugo Mill.	reptant	Rubiaceae	1	0	0	0	0	1	1	2	1	2
Geranium pratense L.	semirosulate	Geraniaceae	1	0	0	1	0	1	0	2	5	2
Glechoma hederacea L.	reptant	Lamiaceae	1	0	0	0	0	1	2	1	3	2
Heracleum sphondylium L.	semirosulate	Apiaceae	1	1	0	0	1	2	2	3	1	2
Knautia arvensis (L.) J.M. Coult	semirosulate	Dipsaceae	1	0	0	1	2	1	2	1	1	2
Leontodon autumnalis L.	rosulate	Asteraceae	1	0	0	0	1	1	1	1	2	1(2)
Leontodon hispidus L.	rosulate	Asteraceae	1	0	0	0	0	0	3	2	2	0(2)
Leucanthemum vulgare Lam.	semirosulate	Asteraceae	1	0	0	0	1	1	2	2	0	2
Pimpinella major (L.) Huds.	semirosulate	Apiaceae	1	0	0	0	0	1	0	1	1	2
Plantago lanceolata L.	rosulate	Plantaginaceae	1	2	1	2	3	1	1	1	2	2
Plantago media L.	rosulate	Plantaginaceae	1	1	0	0	2	1	2	1	3	2
Primula veris L.	rosulate	Primulaceae	1	0	0	1	0	2	3	1	1	1(2)
Prunella vulgaris L.	reptant	Lamiaceae	1	0	0	3	1	1	0	1	2	2
Ranunculus acris L.	semirosulate	Ranunculaceae	1	1	1	0	0	2	0	2	1	2
Ranunculus repens L.	reptant	Ranunculaceae	1	0	1	0	1	0	1	1	4(5)	1(2)
Rumex acetosa L.	semirosulate	Polygonaceae	1	0	0	0	0	0	0	1	3	2
Taraxacum officinale Wiggers	rosulate	Asteraceae	1	1	0(1)	0	1	2	2	1	4	2
Tragopogon pratensis L.	semirosulate	Asteraceae	1	0	0	0	1	0	0	0	1	1(2)
Veronica chamaedrys L.	reptant	Scrophulariaceae	1	0	0	0	0	2	2	1		2

Table2: Overview of functional traits investigated on aboveground plant organs in this study

Trait	Association	Unit	Description
Growth height	light	cm	Growth height of a module measured in the field
Leaf length	light	cm	Mean of the three longest leaves per module
Specific leaf area	light	$mm^2 mg^{-1}$	Leaf area per leaf dry mass
Leaf $\delta^{13}C$	light	‰	^{13}C isotopic signature of leaves
Leaf $\delta^{15}N$	nitrogen	‰	^{15}N isotopic signature of leaves
Biomass:N ratio	nitrogen	$g\ N\ g^{-1}_{dw}$	Unit nitrogen per unit module biomass
Mass-based leaf nitrogen	nitrogen	$mg\ N\ g^{-1}_{dw}$	Nitrogen mass per leaf dry mass
Module biomass		mg	Aboveground dry mass per module
Internode length	reproduction	cm	Mean of 3-5 central internodes per modules main axis
Stem diameter	reproduction	cm	Stem diameter measured in the middle of the main axis
Inflorescence mass fraction	reproduction	$mg\ mg^{-1}$	Inflorescence dry mass per module dry mass
Stem:leaf ratio	reproduction	$mg\ mg^{-1}$	Stem dry mass per leaf dry mass

Table 3: Summary of mixed-effects model analyses of non-reproductive traits combining all vegetative modules from two harvest campaigns and all reproductive modules from the campaign, where flowering phenology of the respective species was most advanced. Models were fitted by stepwise inclusion of terms with the full random effects and subsequent stepwise exclusion of random effect terms. Listed are the results of likelihood ratio tests that were applied to assess model improvement, for (a) computations without and (b) computations with inclusion of the phylogenetic correlation matrix.

	Height					Mean leaf length					Specific leaf area				
		vegetative		reproductive			vegetative		reproductive			vegetative		reproductive	
	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)
Growth form (GF)	2	15.64***	10.54**	27.2***	27.56***	2	21.79***	15.32**	3.89	1.18	2	10.62**	8.72*	10.64**	5.66
Spechies richness (SR)	1	17.6***↑	10.6**↑	13.27***↑	4.58*↑	1	9.56**↑	1.26	11.58***↑	0.68	1	33.65***↑	9.98**↑	7.37**↑	3.24
Legumes (Leg)	1	46.88***↑	44.46***↑	16.03***↑	15.34***↑	1	22.44***↑	19.74***↑	19.26***↑	16.72***↑	1	13.73***↑	13.76***↑	2.79	3.04
GF x SR	2	1.003	0.7	2.4	1.7	2	4.23	4.72	7.47*	4.96	2	1.09	1.46	7.85*	8.04*
Harvest time	1	91.461***	88.84***	NA	NA	1	61.83***	57.28***	NA	NA	1	66.47***	64.26***	NA	NA
Species identity (SpI)	1	300.44***	NA	105.63***	NA	1	103.29***	NA	96.98***	NA	1	220.86***	NA	47.96***	NA
SpI[SR]	2	2.51	0.12	2.03	0.6	2	27.3***	18.74***	6.44*	14.68***	2	0.005	3.18	0.02	0.02

	Leaf $\delta^{13}C$					Leaf $\delta^{15}N$					Biomass: N ratio				
		vegetative		reproductive			vegetative		reproductive			vegetative		reproductive	
	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)
Growth form (GF)	2	7.58*	6.14*	6.94*	8.06*	2	3.08	7.86*	5.03	4.88	2	7.17*	6.54*	2.87	3.12
Spechies richness (SR)	1	17.74***↓	2.54	27.11***↓	6.1*↓	1	3.91*↓	0.52	7.54**↓	5.34*↓	1	1.26	0.14	0.14	0.12
Legumes (Leg)	1	6.47*↓	3.74	10.44**↓	11.08***↓	1	1.16	0.16	0.81	0.84	1	15.51***↑	14.66***↑	4.59*↑	3.9*↑
GF x SR	2	5.34	2.46	15.02***	8.62*	2	1.69	1.84	0.39	0.46	2	0.86	1.14	2.32	1.38
Harvest time	1	18.38***	18.4***	NA	NA	1	2.82	3.36	NA	NA	1	175.24***	174.42***	NA	NA
Species identity (SpI)	1	138.03***	NA	131.31***	NA	1	73.48***	NA	10.48**	NA	1	44.78***	NA	77.26***	NA
SpI[SR]	2	18.52***	5.46	0.13	0.34	2	36.6***	40.38***	2.57	1.34	2	4.99	3.6	4.11	0.06

	Mass-based Leaf N					Module mass				
		vegetative.		reproductive.			vegetative.		reproductive.	
	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	loglike(a)	loglike(b)
Growth form (GF)	2	4.7	6.7*	2.69	4.32	2	16.19***	26.88***	16.1***	23.24***
Spechies richness (SR)	1	1.72	0.12	0.57	0.32	1	0.14	0.16	5.45*↑	0.88
Legumes (Leg)	1	16.83***↑	15.78***↑	6.77**↑	6.8**↑	1	18.91***↑	14.38***↑	11.51***↑	9.56**↑
GF x SR	2	3.58	4.72	3.64	3.68	2	0.23	0.7	2.92	2.7
Harvest time	1	164.23***	163.34***	NA	NA	1	3.83	3.46	NA	NA
Species identity (SpI)	1	45.71***	NA	48.8***	NA	1	209.99***	NA	191.89***	NA
SpI[SR]	2	4.95	3.46	0.06	0.02	2	24.6***	5.32	0	3.96

Table 4: Summary of mixed-effects model analyses of traits characterizing reproductive modules. Models were fitted by stepwise inclusion of terms with the full random effects and subsequent stepwise exclusion of random effect terms. Listed are the results of likelihood ratio tests that were applied to assess model improvement, (a) computations without and (b) computations with inclusion of the phylogenetic correlation matrix.

	Mean internode length (mll)			Stem diameter (StØ)			Inflorescence mass fraction (IMF)			Stem:leaf ratio (SLR)		
		reproductive			reproductive			reproductive			reproductive	
	df	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)	df	loglike(a)	loglike(b)
Growth form (GF)	2	11.433***	4.3	2	19.62***	23.68***	2	5.13	14.24***	2	27.72***	32.64***
Spechies richness (SR)	1	2.22	0.8	1	2.46	0.42	1	3.77	0.48	1	0.37	0.0
Legumes (Leg)	1	14.74***↑	11.78***↑	1	10.76**↑	7.52**↑	1	3.65	3.56	1	4.63*↑	4.16*↑
GF x SR	2	0.34	0.18	2	2.83	2.12	2	1.09	0.2	2	0.91	0.28
Species identity (Spl)	1	52.38***	NA	1	116.69***	NA	1	96.03***	NA	1	47.79***	NA
Spl[SR]	2	1.02	0.08	2	0.19	1.3	2	3.72	8.44*	2	10.02**	1.42

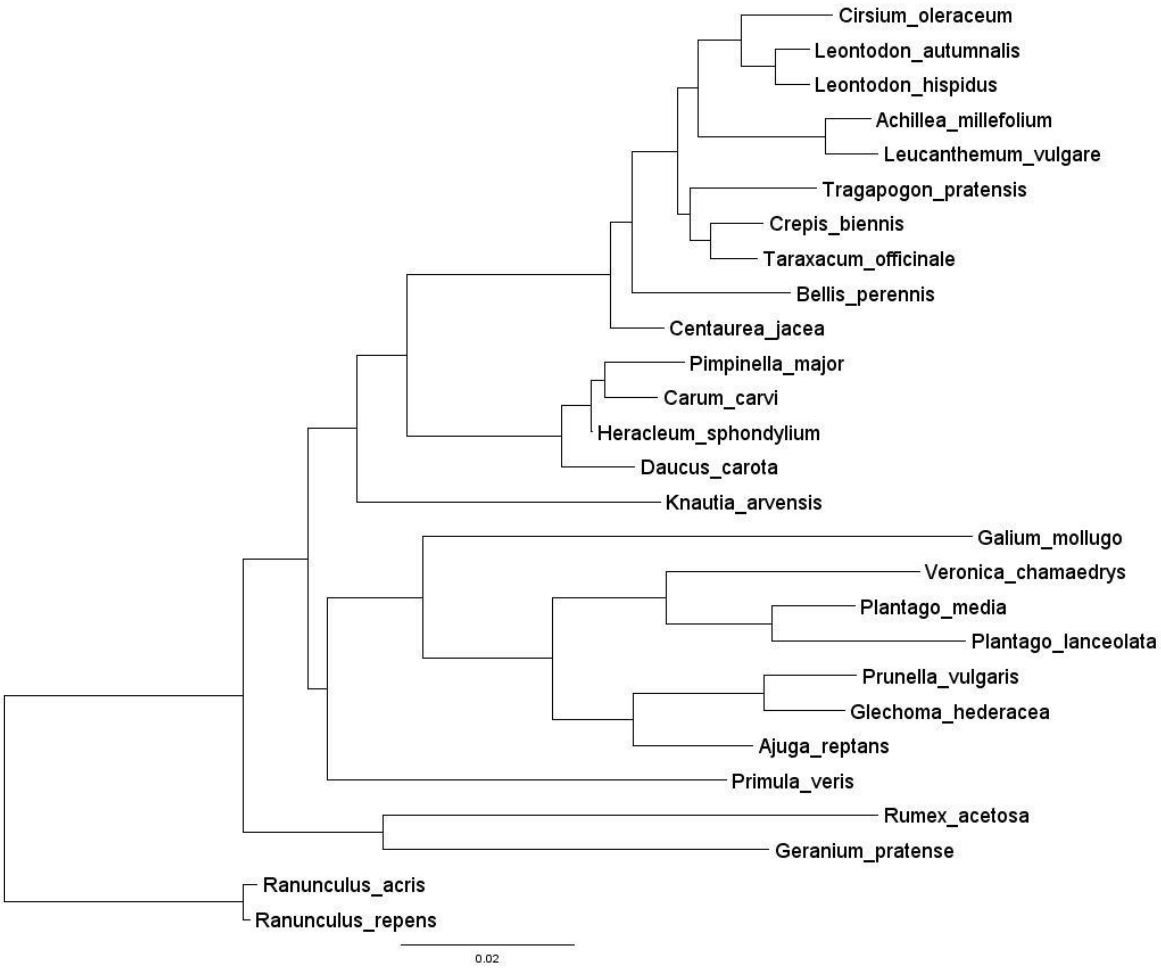

Fig. 1: Phylogenetic relationships among the experimental species.

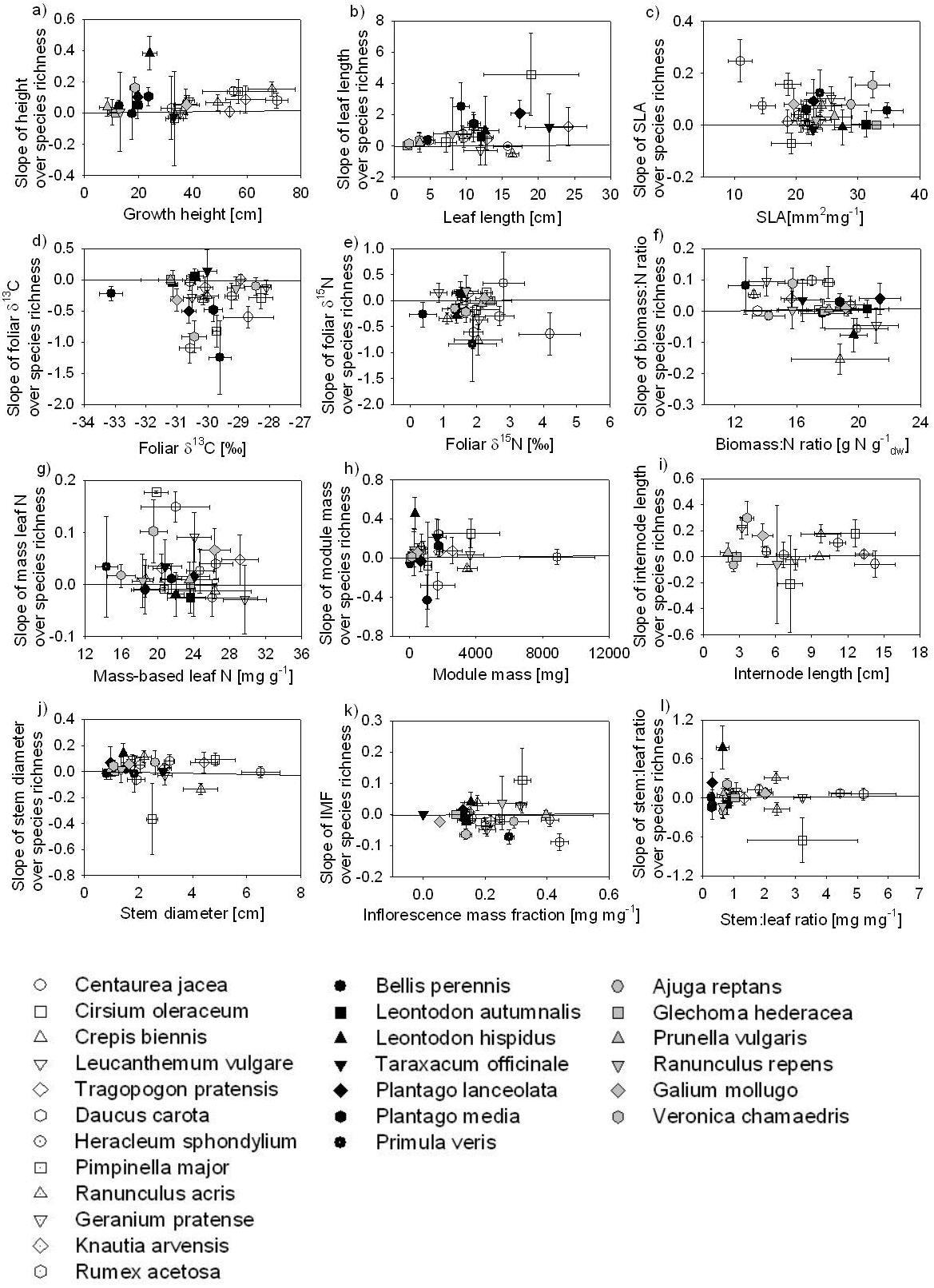

Fig. 2: Mean trait values (± SE) of reproductive modules (averaged across plots) at time of maximum flowering phenology plotted against regression slope (± SE) of trait values against species richness based on the model shown in Table 2. Cases below zero indicate negative trait value-species richness relationships, and cases above zero indicate positive trait values-species richness relationships.

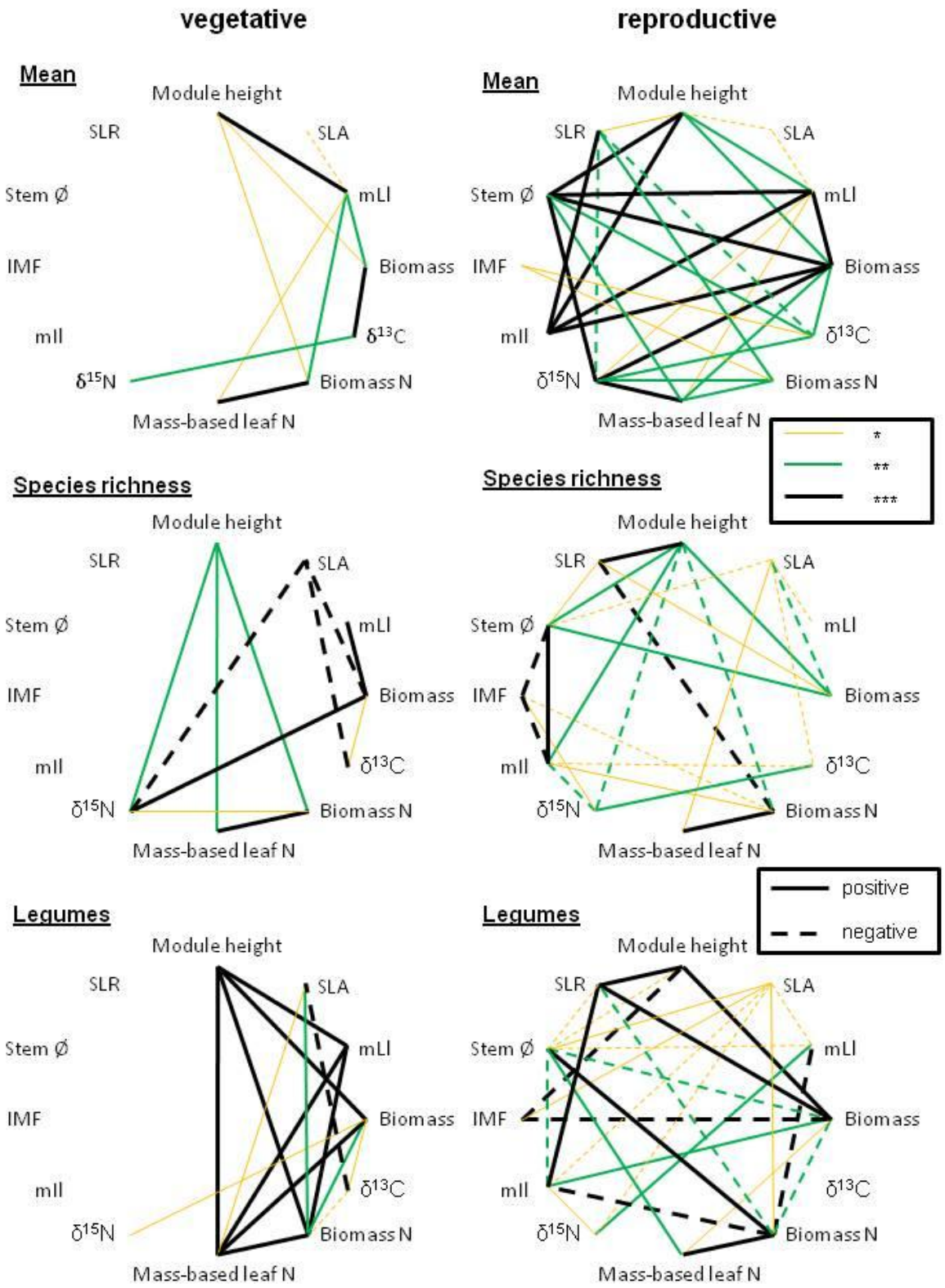

Fig. 3: Correlations for vegetative and reproductive modules among mean trait values (a, b), regression slopes against species richness (c, d) and residual regression slopes against legume presence (e, f). Solid lines indicate positive correlations, dashed lines show negative correlations. Levels of significance are indicated with thick black lines for $P \leq 0.001$, medium green lines for $P < 0.010$, thin orange lines for $P < 0.050$

Appendix

The R code for statistical analysis with mixed-effects models

We used R 2.11.1 (R Development Core Team 2009) and the R package *lme4* (Bates & Sakar 2006) for data analyses.

Abbreviations of variable names are:

block	Experimental blocks of the biodiversity experiment
plot	Experimental plots of the biodiversity experiment
SR	Species richness of the experimental plots of the biodiversity experiment
Leg	Legume presence of the experimental plots of the biodiversity experiment
species	species identity
GF	Growth form of the different species
Fam	Plant family of the different species
harvest	harvest time of the plant modules

Maximum model without phylogenetic correction

```
m0<-
lmer(log(X)~1+(1|plot)+(1+log(SR)|species),REML=F)
m1<-
lmer(log(X)~GF+(1|plot)+(1+log(SR)|species),REML=F)
m2<-
lmer(log(X)~ GF+log(SR)+(1|plot)+(1+log(SR)|species),REML=F)
m3<-
lmer(log(X)~GF+log(SR)+Leg+(1|plot)+(1+log(SR)|species),REML=F)
m4<-
lmer(log(X)~GF+log(SR)+Leg+GF:log(SR)+(1|plot)+(1+log(SR)|species),
REML=F)
m5<-
lmer(log(X)~GF+log(SR)+Leg+GF:log(SR)+harvest+(1|plot)+(1+log(SR)|species),
REML=F)
anova(m0,m1,m2,m3,m4,m5)
```

effects starting with the maximum model (m5)

```
m5<-
lmer(log(X)~GF+log(SR)+Leg+GF:log(SR)+harvest+(1|plot)+(1+log(SR)|species),
REML=F)
m5a<-
lmer(log(X)~GF+log(SR)+Leg+GF:log(SR)+harvest+(1|plot)+(1|species),REML=F)
m5b<-
lmer(log(X)~GF+log(SR)+Leg+GF:log(SR)+harvest+(1|plot),REML=F)
anova(m5,m5a,m5b)
```

Model with phylogenetic correction

```
### load phylogenetic correlation matrix
load("corMatrix.RData")
tree.cor.mat.sorted <-
tree.cor.mat[order(row.names(tree.cor.mat)),order(row.names(tree.cor.mat))]
G.species <- matrix(as.numeric(tree.cor.mat.sorted), 27,27)

### function to directly maximize the loglikelihood-function
### random effects: (1|Plot)+(1|Specie),  species correlated
### we start with a fitted lmer-object with a random effect structure as
  close as possible,
### i.e., independent species effects
### the second argument increases the diagonal values of the covariance
  matrix to achieve positive definitness

lmer.corr <- function(lmerfit, factor)
{

### extract design matrix for fixed effects
X <- as.matrix(lmerfit@X)
n <- nrow(X)
### design matrix random effects
Zt <- as.matrix(lmerfit@Zt)
### response vector
y <- as.vector(lmerfit@y)

### starting values for likelihood maximization from almost correct model
### starting values cannot be on the boundary - add a tiny number
fixef.start <- fixef(lmerfit)
sigma2.plot <- as.numeric(VarCorr(lmerfit)[[1]])+0.00001
sigma2.species <- as.numeric(VarCorr(lmerfit)[[2]])+0.00001
sigma2.eps <- factor*summary(lmerfit)@sigma^2

start <- c(sigma2.plot, sigma2.species,sigma2.eps, fixef.start)

### definition of loglikelihood-function
likelihood <- function(params)
{
### covariance matrix of random effects
G.s <- params[2] * G.species
G <- bdiag(diag(params[1],length(unlist(ranef(lmerfit)[[1]]))),G.s)

### residual error variance
R <- diag(params[3],n)

### marginal covariance matrix
V <- R+ t(Zt) %*% G %*% Zt

### beta
beta <- params[4:length(params)]

### mean
mu <- as.vector(as.numeric(X %*% beta))

### - log-likelihood
as.numeric(n/2*log(2*pi)+determinant(V, logarithm=T)$modulus/2+ t(y-mu) %*%
solve(V) %*% (y-mu)/2)
}

constrOptim(start,likelihood, ui=cbind(diag(3),
matrix(0,nrow=3,ncol=length(start)-3)),ci=rep(0,3), mu=0.001,grad=NULL)
```

```
}

fit <- function(formula)

m<-lmer(formula,data=veg,REML=F,na.action=na.exclude)
factor <- 1
repeat
 {
 x <- try( result <- lmer.corr(m,factor),silent=T)
 if (!inherits(x, "try-error")) break
 factor <- factor+1
 }
cat(as.character(formula)[c(2,1,3)],"   ",as.numeric(m@dims["p"]),"   ",
result$value, "   ", 2*result$value+2*as.numeric(m@dims["p"]+3),"\n")
 }

### example fit

fit(d13C~wuchs+log(Div)+Leg+(1|Plot)+(1|Specie))
```

Table A1: Name of experimental species where the *rbc*L gene sequences was missing in the database and the relevant closely related used species.

Study species	**Closely related species**
Carum_carvi L.	*Aegopodium podagraria* L.
Centaurea_jacea L.	*Centaurea melitensis* L.
Cirsium_oleraceum (L.) Scop.	*Cirsium arvense* (L.) Scop.
Crepis_biennis L.	*Youngia japonica* (L.) DC.
Geranium_pratense L.	*Geranium divaricatum* EHRH.
Knautia_arvensis (L.) J.M. Coult	*Knautia intermedia* Pernh. & Wettst.
Leontodon_autumnalis L.	*Hypochaeris radicata* L.
Pimpinella_major (L.) Huds.	*Pimpinella saxifraga* L.
Ranunculus_repens L.	*Ranunculus cantoniensis* DC.
Veronica chamaedrys L.	*Veronica persica* POIR.

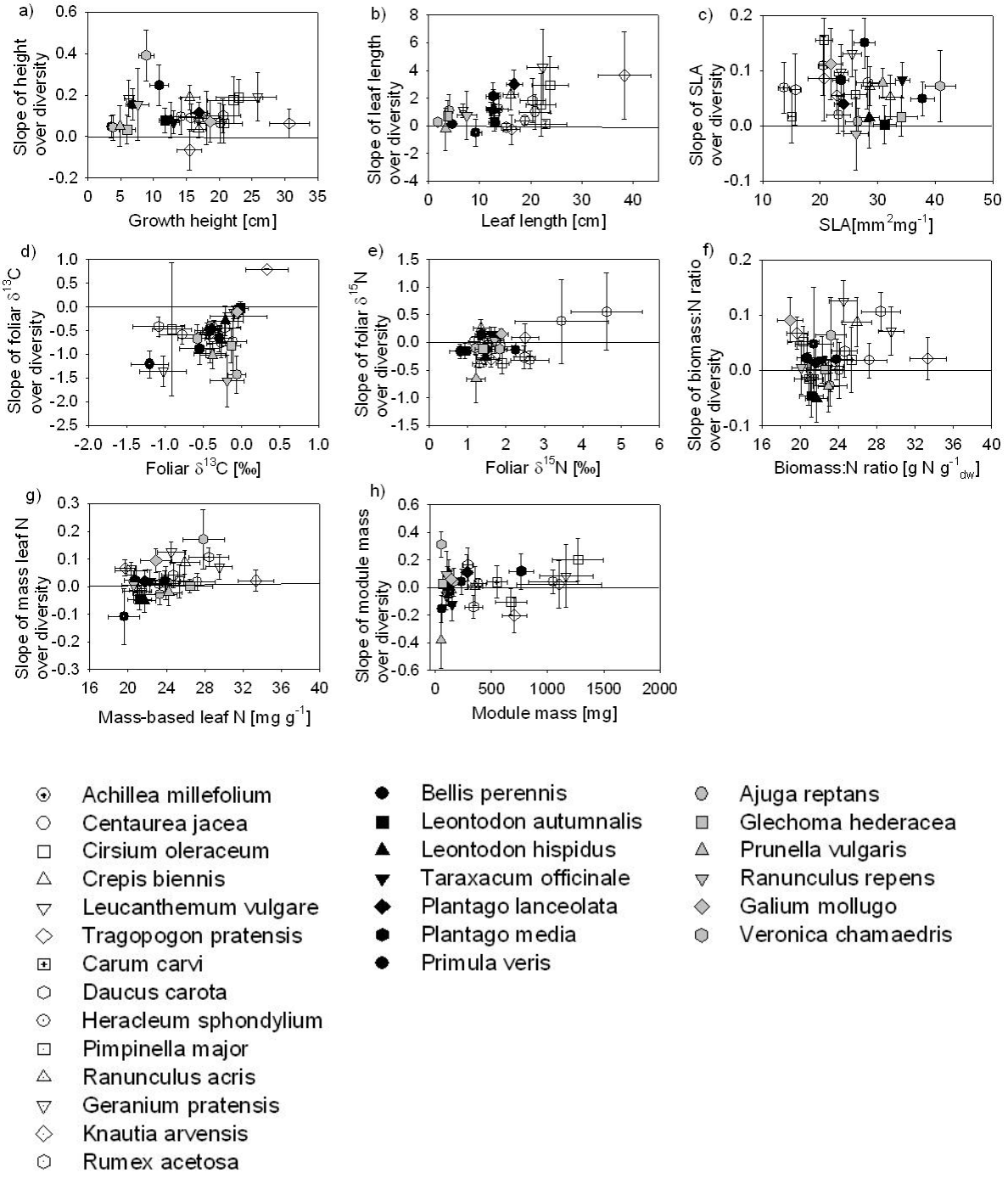

Fig. A1: Mean trait values (± SE) of vegetative modules (averaged across plots and season) plotted against regression slope (± SE) of trait values against species richness based on the model shown in Table 2. Cases below zero indicate negative trait value-species richness relationships and cases above zero indicate positive trait value-species richness relationships.

Chapter 2

Selection for monoculture and mixture genotypes in a biodiversity experiment

Annett Lipowsky, Bernhard Schmid & Christiane Roscher (2011)

Basic and Applied Ecology in press.

Abstract

Studies in experimental grasslands have shown variation in plant individual performance in response to neighbourhood diversity. To which extent these responses are due to phenotypic plasticity or genetic variation is largely unknown. We collected seed families of five herbaceous species (*Cirsium oleraceum*, *Crepis biennis*, *Plantago lanceolata*, *Plantago media* and *Rumex acetosa*) in monocultures and 60-species mixtures 5 years after establishment and replanted or transplanted the offspring into the same monocultures and 60-species mixtures. In all five species the actual environment significantly affected plant survival, growth and performance in terms of shoot biomass and investment into reproduction, indicating stronger competition for light and different levels of herbivory in mixtures as compared with monocultures. Effects of the original environment were smaller and less consistent, but indicated differential selection in monocultures vs. mixtures. The interaction between actual and original environment, corresponding to the "home" vs. "away" comparison, was rarely significant, yet this was providing a first sign of local adaptation. We conclude that, for the investigated plant species, more than five growing seasons in monocultures or mixtures would be needed to better demonstrate the selection of genotypes specifically adapted to monocultures or mixtures. A faster local adaptation may have been prevented by the ability of these species to respond to variation in neighbourhood diversity to a large degree via phenotypic plasticity and other factors.

Zusammenfassung

Untersuchungen in experimentellen Graslandbeständen haben gezeigt, dass die Wuchsleistung von Pflanzenindividuen in Abhängigkeit von der Diversität benachbarter Pflanzen variiert. Ob diese Reaktion auf phänotypische Plastizität oder genetische Variation zurückzuführen ist, ist bisher nicht geklärt. Wir sammelten in Monokulturen und 60-Arten-Mischungen 5 Jahre nach ihrer Etablierung Samenfamilien von fünf krautigen Arten (*Cirsium oleraceum*, *Crepis biennis*, *Plantago lanceolata*, *Plantago media* und *Rumex acetosa*) und pflanzten die Nachkommen in einem reziproken Verpflanzungsexperiment in ihre eigenen Monokulturen und die 60-Arten-Mischungen. Bei allen fünf Arten hatte die aktuelle Umwelt großen Einfluss auf die Ausprägung der untersuchten Merkmale, die eine stärkere Konkurrenz um Licht in Mischungen im Vergleich zu Monokulturen und einen unterschiedlichen Herbivorendruck anzeigten. Artabhängig beeinflusste die aktuelle Umwelt das Überleben, die Sprossbiomasse und die Investition in Reproduktion bei den verpflanzten Individuen. Effekte der Herkunft waren geringer und weniger konsistent, so dass die Anzeichen für eine unterschiedliche

Selektion und daraus resultierende genetische Variation nur gering waren. Lokale Anpassungen, die zu einer höheren Leistungsfähigkeit der Nachkommen von Samenfamilien führen, die in ihre Herkunftsumwelt zurückverpflanzt wurden im Vergleich zu solchen, die zwischen Monokulturen und Mischungen verpflanzt wurden, waren selten festzustellen, gaben aber erste Hinweise auf eine lokale Adaptation. Aus unserem Experiment folgern wir, dass für die untersuchten Pflanzenarten mehr als 5 Jahre Selektionsdruck durch eine Umwelt mit unterschiedlicher pflanzlicher Diversität notwendig ist, um besser eine Selektion von Genotypen, die speziell an Monokulturen oder Mischungen angepasst sind, nachzuweisen. Dieses Ergebnis kann dadurch bedingt sein, dass die untersuchten Arten in beträchtlichem Maß durch phänotypische Plastizität auf eine Variation der pflanzlichen Diversität in ihrer Nachbarschaft reagieren können.

Keywords: genetic variation, local adaptation, mixture, monoculture, phenotypic plasticity, reciprocal transplant experiment

Introduction

Genetic differentiation among natural plant populations of the same species may reflect adaptation in response to their local environment (Linhart & Grant 1996). Local adaptive genetic variation in single plant species has been reported at a small scale (e.g. Turkington & Harper 1979, Antonovics & Primack 1982, Schmid 1985, van Tienderen 1990) or across larger geographic distances (e.g. Joshi, Schmid, Caldeira, Dimitrakopoulos, Good et al. 2001, Santamaría, Figuerola, Pilon, Mjelde, Green et al. 2003, Becker, Colling, Dostal, Jakobsson & Matthies 2006, Bischoff, Crémieux, Šmilauerová, Lawson, Mortimer et al. 2006). Given their sessile life-form and low genetic mobility via seeds and pollen strong genetic differentiation in response to persistent small-scale variation in the abiotic environment can be expected (e.g. Antonovics 1968, Ducousso, Petit, Valero & Vernet 1990, Antonovics 2006). However, small-scale variation in the biotic environment of plants may be more dynamic. Therefore, it raises the question whether variation in the biotic environment also leads to a genetic differentiation of populations in an ecologically relevant time scale (Turkington & Harper 1979, Linhart 1988) or whether phenotypic plasticity is more important for species adjustment to more heterogeneous biotic environments (Schlichting 1986, Sultan 1987, Schmid 1992).

Reciprocal transplant experiments can be used to separate genetic variation from phenotypic plasticity and to test for plant adaptations to local environmental conditions (Antonovics & Primack 1982, Schmid 1985). If plants grown in the same environment differ from each other according to their genotype (population origin), they express genetic variation. If plants of the same genotype differ from each other if grown in different environments, they express phenotypic plasticity. Finally if there is an interaction between genotype and environment in the way that plants transplanted to “home” sites have an advantage over plants transplanted to “away” sites, they can be considered as locally adapted (Schmid 1992, Joshi et al. 2001, Kawecki & Ebert 2004). An advantage of reciprocal transplant experiments to test for local adaptation is that the selection pressures driving the adaptation need not to be known (Schmid 1992). To find out which drivers may be responsible, ecological differences between the different types of local environments can be examined (Raabová, Münzbergová & Fischer 2007, Bowman, Perret, Hoehn, Galeuchet & Fischer 2008). In natural environments, this approach can provide hypotheses about potential selection pressures to be tested *a posteriori* in subsequent experiments. Here we used neighbourhood diversity as an *a priori* selection pressure and investigated if test species responded to this selection pressure by genetic differentiation and local adaptation or if they could adjust to varying neighbourhood diversities by phenotypic plasticity.

Our study was motivated by the observation of positive plant diversity–productivity relationships in grassland biodiversity experiments (Hooper, Chapin, Ewel, Hector, Inchausti et al. 2005, Balvanera, Pfisterer, Buchmann, He, Nakashizuka et al. 2006, Cardinale, Srivastava, Duffy, Wright, Downing et al. 2006). In spite of this overall increase of community performance, responses of individual species to altered community diversity vary greatly (Dimitrakopoulos & Schmid 2004, Roscher, Schumacher, Weisser, Schmid & Schulze 2007, Marquard, Weigelt, Roscher, Gubsch, Lipowsky et al. 2009) suggesting that plants of any particular species may respond to conspecific neighbours differently than to heterospecific neighbours. It is not known so far to which extent these responses are due to selection between different plant genotypes or to phenotypic plasticity of individual genotypes.

Several previous studies already investigated effects of plant diversity on individual plant performance in experimental grasslands. They either used individuals which were members of the sown resident community (Dassler, Roscher, Temperton, Schumacher & Schulze 2008, Thein, Roscher & Schulze 2008) or planted so-called "phytometers" into the resident communities (Diemer & Schmid 2001, Scherber, Milcu, Partsch, Scheu & Weisser 2006a, Mwangi, Schmitz, Scherber, Roscher, Schumacher et al. 2007). These studies, however, could only measure the phenotypic response of the plants to the different actual environments and not test for effects of different plant origin. To disentangle the influence of plant origin and actual environment, we used plants of different origin (with the original environment as monoculture or 60-species mixture) and transplanted or replanted them into actual environments of the same type. Rates of genetic differentiation depend on generation times and the pollination system of plant species (Linhart & Grant 1996), where a short life-cycle and self-compatibility are likely to promote genetic differentiation. The majority of plant species in temperate grasslands is perennial and can reproduce via clonal growth. In spite of large differences in their mating system including apomixis, complete self-pollination and mixed mating systems (Klotz, Kühn & Durka 2003), genetic differentiation may thus mainly depend on differential mortality and growth between clones originally sown to establish grassland. We allowed for such genetic differentiation to take place over five growing season in monocultures and 60-species mixtures in our experiment. Then we reciprocally transplanted plants between these two environments. We used five herbaceous species as model system, namely *Cirsium oleraceum* (L.) Scop., *Crepis biennis* L., *Plantago lanceolata* L., *P. media* L. and *Rumex acetosa* L. We tested if these species (1) showed evidence for genetic differentiation between different original environments when grown in the same

actual environment, (2) showed evidence for phenotypic plasticity between different actual environments when taken from the same original environment, and (3) showed evidence for local adaptation to their home environments when reciprocally transplanted and replanted between original and actual environment.

Material and methods

FIELD SITE: THE JENA EXPERIMENT

The Jena Experiment is a large biodiversity experiment situated in the floodplain of the river Saale near Jena (Germany, 50°55' N, 11°35' E, 130 m a.s.l.). Mean annual temperature is 9.3 °C, and mean annual precipitation amounts to 587 mm (Kluge & Müller-Westermeier 2000). The experiment was set up using a pool of 60 plant species commonly occurring in species-rich Central European, semi-natural grasslands (*Arrhenatherion* communities, Ellenberg 1988). A complete list of the experimental species is provided in Appendix A. The experiment includes the 60-species mixture (4 replicates) and monocultures of several of the 60 species on 20 × 20 m plots. The monocultures were not replicated on these large plots but for each of the 60 species two replicated monocultures were also sown on small plots of 3.5 × 3.5 m (for details see Roscher, Schumacher, Baade, Wilcke, Gleixner et al. 2004). All plots were sown in May 2002, were mown twice a year (early June, early September) and did not receive fertilizer addition. To maintain only the originally sown species composition, plots were weeded in biannual weeding campaigns (early April, middle of July).

STUDY SPECIES

Five herbaceous species were selected for the reciprocal transplant experiment, *Crepis biennis* (Asteraceae), *Cirsium oleraceum* (Asteraceae), *Plantago lanceolata* (Plantaginaceae), *Plantago media* (Plantaginaceae) and *Rumex acetosa* (Polygonaceae). The chosen species form a rosette after germination and individual genets remain distinguishable for a long time, which is important to reduce the danger of sampling clonal replicates of single genets in the transplant study. *Crepis biennis* is a biennial to monocarpic perennial species, i.e. plant individuals die off after flowering, while the other four species are perennial herbs. Although the chosen species typically grow in extensively managed grasslands, *P. lanceolata*, *C. biennis* and *R. acetosa* also frequently occur in disturbed ruderal habitats (Rothmaler 2002). *Plantago lanceolata* and *P. media* are wind- or insect-pollinated, protogynous and

incompletely self-incompatible, i.e. selfing may occasionally occur (Sagar & Harper 1964). The dioecious species *R. acetosa* is predominantly wind-pollinated. *Crepis biennis* is self- or insect-pollinated. Self-compatibility has been rarely studied in the genus *Crepis*, but so far no obligate selfer is known (Enke, Fuchs & Gemeinholzer 2011). Apomixis has also been reported in *C. biennis* (Rothmaler 2002). *Cirsium oleraceum* is insect-pollinated and the only study species for which frequent self-compatibility has been described (Bureš, Šmarda, Rotreklová, Oberreiter, Burešová et al. 2010).

RECIPROCAL TRANSPLANT EXPERIMENT

In summer 2006, seeds from fruiting plants of the five study species were collected in small and if available in large monocultures and in three replicates of the 60-species mixture. We collected seeds in monocultures from four mother plants of *C. oleraceum* (one large and one small plot), four mother plants of *R. acetosa* (one small plot), five mother plants of *C. biennis* (one small plot), six mother plants of *P. lanceolata* (one large plot) and six mother plants of *P. media* (two small plots). The same number of seed families (fruits) for each species was collected in the 60-species mixture (three large plots). Seeds were stored at –20 °C to maintain their viability until the start of the experiment. From each of the 50 (= (4 + 4 + 5 + 6 + 6) × 2 diversity levels) seed families the mass of 50 seeds (or a smaller number if fewer seeds were available) and the germination rate of 20 seeds were determined in January 2007. Germination tests were performed in Petri dishes on moistened filter paper in a climate chamber under standardized light and temperature conditions (16 h light at 20 °C, and a night temperature of 12 °C). The original environment (monoculture vs. mixture) and the plot of origin did not affect germination rates of the seed families in all studied species except for reduced germination rates of *C. oleraceum* collected in mixture plots (analyses not shown). Seedlings from each seed family were planted into QuickPot™ trays (cylindrical pots with a volume of 0.2 L, Herku-Plast, Germany) in soil from the field site and grown in an unheated glasshouse. The young plants were watered every second day.

From 1–6 June 2007, plants were transplanted and replanted into the plots of the biodiversity experiment. At this time, we found no effect of the original environment (monoculture or 60-species mixture) on leaf number and leaf length of plantlets of our study species, with the exception for leaf number in *C. biennis*. However, differences among seed families were often significant, with exception of *C. oleraceum* (analyses not shown). Thus, effects of maternal environment or genetic differentiation dependent on the original environment were near negligible for the early stages of development of the transplants. One

half of the offspring of each seed family were transplanted to their own monoculture plots and the other half to 60-species mixture plots (in total 16-18 plants per seed family, i.e. 8 or 9 plants per actual environment). This procedure covered all possible combinations of original environment × actual environment: monoculture seed families transplanted into mixtures, monoculture seed families replanted into monocultures, mixture seed families transplanted into monocultures and mixture seed families replanted into mixtures. In monoculture plots, the offspring of different seed families of the respective monoculture species was randomly distributed within a subplot of 1.00 × 1.25 m in a regular grid with a between-plant distance of 0.25 m (one small plot per species). In mixture plots, the offspring of different seed families and species was completely randomly distributed in subplots of 3.5 × 2.5 m in a regular grid with a between-plant distance of 0.25 m using the three large plots of the 60-species mixture. In total, 1016 plantlets were transplanted and replanted and marked with plastic labels of different colours next to the plants to ease identification during data collection.

All plants were harvested on 26–30 May 2008. Canopy height of the surrounding vegetation, plant height, maximum leaf length, number of leaves and leaf damage by herbivores were recorded. Leaf damage was estimated visually as percentage loss of leaf area, from 1–10 in single steps and from 10–100 in steps of 5%. Most of the harvested plants were vegetative (72%), but in *C. biennis* 73% of all plants flowered. Thus, numbers of inflorescences according to their stage of development (bud-forming, flowering or fruiting) were counted only for *C. biennis*. Leaves, stems (including branches) and inflorescences were dried separately for 48 h at 70 °C and weighed. Relative height was calculated as the quotient of plant height by canopy height of the surrounding vegetation.

STATISTICAL ANALYSIS

Generalized Linear Models (GLM) with a normal distribution of errors and a linear link function were applied to get analyses of variance tables for continuous variables (e.g. plant height, biomass, maximum leaf length), count data (leaf number) and percentage of leaf damage. If necessary, response variables were log-transformed prior to analyses to meet assumptions of normality and homoscedasticity. Generalized Linear Mixed Models (GLMM) with a binomial error distribution using a complementary log-log link structure were used for data on survivorship. A logit link structure was applied for binary data on transition into the reproductive stage. Results from GLMMs are presented in analysis of deviance tables. Effects of species and maternal plant identity, original environment (environment where maternal

plants came from: monoculture vs. mixture) and plot of origin, actual environment (environment where seed offspring of maternal plants grew: monoculture vs. mixture) and plot of actual environment and interactions were included as explanatory terms. Of particular interest among the interactions were original × actual environment interactions, which can be viewed as "home" vs. "away" contrasts (home: original environment = actual environment, away: original environment ≠ actual environment). Species identity, original environment, species identity × original environment interactions and plot of the seed family were tested against seed family as error, because each seed family could only include plants of the same species, original environment and plot of the seed family (nested structure). The remaining terms were tested against residuals. Due to the large differences among species, we also analyzed data for each species separately, using the model mentioned above without species identity effects. GLMMs were calculated with Genstat, version 13.1 (VSN International Ltd.). The statistical software R (Version 2.7.2, http://www.r-project.org) was used for all other calculations and analyses.

Results

Species identity had significant effects on all measured traits (Table 1) and explained a large proportion of variation. The actual environment in which the plants were growing during the experiment also significantly affected survival of transplants and the majority of plant traits, except for the number of leaves and proportion of transplants reaching the reproductive stage (Table 1, Fig. 1). However, significant species identity × actual environment interactions in all analyses showed that the potential for phenotypic plasticity varied among species (Table 1).

The original environment had no consistent effects across the five test species. Significant species identity × original environment interactions for plant height and leaf length indicated that the amount of genetic differentiation caused by 5 years of selection differed among the five species. In comparison to the effects of the actual environment, effects of the original environment were small, suggesting that genetic differentiation was less pronounced than phenotypic plasticity in the five species investigated (Table 1). Furthermore, because the original environment × actual environment interactions across species were only significant in the case of leaf damage and inflorescence biomass, local adaptation to home sites across all species was not a general feature in our study. We therefore analyzed a number of traits for each species separately to further test for local adaptation of single species.

CIRSIUM OLERACEUM

Cirsium oleraceum responded to the actual environment by increased plant height and leaf length and reduced leaf number and relative height compared to the surrounding vegetation in mixture (see Appendix A: Table 2, Fig. 2A-B). Plants of mixture origin had higher numbers of leaves than plants of monoculture origin in both actual environments (Fig. 2B), indicating genetic differentiation due to selection by neighbourhood diversity. Furthermore, a significant original environment × actual environment interaction for plant height (see Appendix A: Table 2) suggested some adaptation to the home evironment in this species: plants whose parents had been selected in mixtures for 5 growing seasons were taller in mixtures (home environment) than plants whose parents had been selected in monocultures (away environment), but if the growth place was monoculture the difference between the two origins was very small (Fig. 2A). In contrast, the significant original environment × actual environment interaction for leaf herbivory (see Appendix A: Table 2) was related to more herbivory damage in home than in away environments (Fig. 2D).

PLANTAGO LANCEOLATA

Plantago lanceolata had a higher performance in mixture than in monoculture (see Appendix A: Table 2, Fig. 2F-K). Plants in mixtures grew taller, produced more and longer leaves with a higher biomass per leaf, and achieved a higher plant individual and inflorescence biomass ($F_{1,32}$ = 4.39, p = 0.040). They suffered less leaf herbivory than plants growing in monocultures, while survival and transition into the reproductive stage (analysis not shown) did not depend on our treatments. Origin effects and interactions were small and not significant indicating that in *P. lanceolata* phenotypic plasticity was responsible for an adjustment to environmental variation created by plant diversity. However, maximum leaf length showed significant genetic differences among seed families.

PLANTAGO MEDIA

In *P. media*, significant effects of the actual environment were observed for plant height and leaf length (see Appendix A: Table 2, Fig. 2L). However this phenotypic plasticity was not sufficient to prevent increased light competition (plants reached only 15% of vegetation height). In mixtures a lower proportion of plants reached the reproductive stage ($F_{1,29}$ = 6.63, p = 0.011), which was paralleled by increased leaf herbivory. Plants of mixture origin had decreased height and shorter leaves in both actual environments (Fig. 2L). Significant genetic

differences among seed families were found for plant height, leaf length and leaf number (see Appendix A: Table 2).

RUMEX ACETOSA

Rumex acetosa again showed a similar response to growing in mixture vs. monoculture as the other species (see Appendix A: Table 2, Fig. 2Q-T). Similar to *C. oleraceum*, the increased plant height and maximum leaf length was accompanied by a reduced number of leaves in mixtures. The mixture environment decreased survival of *R. acetosa* transplants (Fig. 2U). Origin effects and interactions were not significant.

CREPIS BIENNIS

Crepis biennis showed similar responses to the actual environment as the previous species (see Appendix A: Table 2). However, in contrast to the other investigated species, most plants of *C. biennis* were reproductive (73%). The proportion of reproductive plants was reduced in the mixture environment (see Appendix A: Table 3). Number of leaves, plant individual biomass and inflorescence biomass were significantly lower in mixture, while maximum leaf length was higher in mixture than in monoculture (see Appendix A: Tables 2 and 3; Fig. 2W-X, 3A). Again, in mixture survival of transplants decreased (Fig. 2Z), while leaf herbivory did not differ among treatments (Fig. 2Y). Plants had fewer leaves and lower inflorescence biomass in home than in away environments (significant original environment × actual environment interactions, see Appendix A: Tables 2 and 3). Different proportions of bud-forming and of flowering and fruiting inflorescences gave some evidence for a postponement of flowering in mixture compared to monoculture (Fig. 3B-D). The significant original environment × actual environment interaction for the proportion of bud-forming inflorescences suggested a later flowering time in home than in away environments especially for plants of mixture origin.

Discussion

In this study we focused on the issue whether variation in a plant's abiotic and biotic environment in plant communities with low and high neighbourhood diversity may cause differential selection of genotypes in a 5-year biodiversity experiment. Our controlled reciprocal transplant experiment with five herbaceous plant species showed that the actual environment had large effects on plant survival, growth characteristics related to plant performance and investment into reproduction, suggesting a large potential of the studied

species for environmentally-induced phenotypic plasticity. Effects of the original environment were smaller and less consistent, but our study did provide first evidence that different neighbourhood diversity can lead to genetic differentiation in plant species and that plants may adapt to their local biotic environment, suggesting the evolution of monoculture and mixture genotypes.

Genetic differentiation between monocultures and mixtures as selection environments

Our study differs from other transplant studies in natural communities in that the environments of origin were experimental treatments and not pre-existing habitats naturally varying in plant species diversity. All material within each test species had the same origin before the populations were subjected to the different selection environments. In previous transplant studies, which found larger effects of origin, natural selection had worked for an unspecified, but presumably much longer time, on plant material that may already have arrived from different sources in the different natural environments (e.g. Becker et al. 2006, Bischoff et al. 2006, Bowman et al. 2008). However, despite the relatively short selection in the different original environments (monocultures vs. mixtures, which had been established from the same seed source) three of five studied species did show indications of genetic differentiation between the two experimental original environments (*C. oleraceum*, *P. media*) or local adaptation (*C. oleraceum*, *C. biennis*; see below).

In *P. media*, plants selected in monocultures grew taller and had a longer maximum leaf length in both actual environments (Fig. 2L). Surprisingly, this was in the opposite direction of phenotypic plasticity. However, in *P. media* with its relatively short stature, tall genotypes may be selected for only in monoculture where they can overtop their con-specific neighbours whereas in mixtures they would not be able to overtop the taller interspecific neighbours. In contrast, *C. oleraceum* plants selected in mixtures produced more leaves (Fig. 2B) and achieved a taller growth in the mixture environment (Fig. 2A). In this tall-statured species, selection for increased leaf number and height is more likely to be adaptive allowing the species to reach the upper canopy layers in mixtures. Nevertheless, this selection might be costly if the increased height and leaf number comes at the expense of reduced defense against herbivores as indicated by higher rates of leaf damage in plants originating from mixtures (see Fig. 2D).

Species which did not show any evidence of genetic differentiation after five seasons of selection in monocultures vs. mixtures were *R. acetosa* and *P. lanceolata*. From previous studies it is known that *P. lanceolata* has a large phenotypic plasticity (Antonovics & Primack

1982) which may have allowed this species to cope with the experimentally imposed variation in neighbourhood diversity during the selection period. Both *R. acetosa* and *P. lanceolata* typically occur in a wider habitat spectrum than the other study species, including disturbed ruderal places. Therefore, a larger phenotypic plasticity may be expected in these species. The capacity for a larger phenotypic plasticity in a species may reduce the rapid evolution of genotypes (which may become mal-adapted if the environment changes frequently; Sultan & Spencer 2002).

PHENOTYPIC PLASTICITY IN RESPONSE TO MONOCULTURES AND MIXTURES AS ACTUAL ENVIRONMENTS

The actual environment in which the plants grew had larger effects on performance (shoot biomass, survival) and other traits than their original environment or seed family identity. Thus, our five study species exhibited a large phenotypic plasticity when they grew in monoculture vs. mixture. In mixtures, plants generally were taller and produced longer leaves than in monocultures. The 60-species mixtures in the Jena Experiment had a taller canopy and higher shoot density than every monoculture of the species investigated here (Dassler et al. 2008, Marquard et al. 2009), and presumably exerted higher light competition. Taller growth due to stem and leaf elongation is a typical response to vegetation shading, initiated by reduced light quantity and changes in light quality with a decrease in the red:far red ratio (Schmitt, McCormac & Smith 1995). If this plastic response allows plants to reach more light, it is adaptive (Callaway, Pennings & Richards 2003). Nevertheless, the reduced relative plant height of all studied species in mixtures (Fig. 1B) suggested that increased height growth may be mal-adaptive if species do not reach the upper canopy layers in mixtures with tall-statured species.

The actual environment also had large effects on the investment into reproduction and the flowering phenology of *C. biennis*. The reduced inflorescence biomass of *C. biennis* in mixtures might be caused by the reduced light availability where species often may survive but reduce their reproductive effort (Chiariello & Gulmon 1991). In most flowering plants of temperate regions the onset of flowering is controlled by photoperiod. However, the response to day length is often to some degree dependent on plant size (Klinkhamer, de Jong & Meelis 1987) and could explain the delayed flowering of the less productive *C. biennis* plants in mixtures.

All our test species except *P. lanceolata* suffered higher leaf damage in mixtures than in monocultures. Increasing leaf herbivory with increasing plant species and functional group

richness was also reported by Mulder, Koricheva, Huss-Danell, Högberg & Joshi (1999) and Scherber et al. (2006a). This increase might have several reasons. Firstly, stronger competition for light in multi-species mixtures might have enhanced plant individual susceptibility to herbivores. Secondly, legumes present in the 60-species mixtures of our experiment (see Appendix A) might have increased herbivory due to a fertilization effect of legumes that increased plant nutritional quality (Scherber, Mwangi, Temperton, Roscher, Schumacher et al. 2006b). The different result obtained with *P. lanceolata* is in line with agricultural experiments suggesting larger herbivore damage at the plant individual level in monocultures than in plant mixtures because of a larger density of specialist herbivores (Andow 1991, Finch & Collier 2000).

LOCAL ADAPTATION VIE THE EVOLUTION OF MONOCULTURE AND MIXTURE GENOTYPESß

Despite the large phenotypic plasticity of the studied species and the relatively short selection period, we found already some evidence for selection at home sites. Plants of *C. oleraceum* grew taller when they were replanted into the environment that was their home for five growing seasons than if they were transplanted to the away environment which they had not experienced for at least five growing seasons. However, the opposite pattern, i.e. a negative home effect was found for herbivory in this species (Fig. 2D). Although it is possible that insects with a shorter generation time causing leaf damage might become locally adapted even faster than perennial plants (Crémieux, Bischoff, Šmilauerová, Lawson, Mortimer et al. 2008), local adaptation of insect herbivores is less probable in our experiment at a relatively small spatial scale because of their high mobility. Therefore, negative genetic correlations with other traits could be responsible for this negative home effect. However, further tests on fitness consequences would be required to conclude on local adaptation with respect to height growth or local mal-adaptation in response to herbivore pressure.

In addition to large phenotypic plasticity, there are other factors which might have prevented rapid local adaptation in our study. First, genetic variation in the original seed material might have been low (this material was purchased from commercial suppliers; Roscher et al. 2004) reducing the chance for selection and adaptation to the biotic environment in different experimental communities and increasing the probability for species loss (Vellend & Geber 2005, Vellend 2006). Second, because of space limitation in the experimental plots we could only use a low number of seed families per origin and grow a small number of offspring per seed family (8 to 9 plants per treatment) lowering the statistical

power to detect small effects of local adaptation. Third, the measured vegetative traits may have been less responsive to selection than other traits associated with their greater inherent variation due to phenotypic plasticity than for example reproductive structures, in particular flowers (Schmid 1992). In addition, plant characteristics such as longevity and mating system have been suggests to affect the evolution of local adaptation. A short life cycle and self-compatibility are likely to increase genetic differentiation at small scale (Hartl & Clark 1989, Linhart & Grant 1996). For instance, only recently Fakheran, Paul-Victor, Heichinger, Schmid, Grossniklaus et al. (2010) have demonstrated that populations of the annual plant species *Arabidopsis thaliana* diverged both phenotypically and genetically after only five generations of selection under different disturbance regimes. In our study, only *C. biennis* is biennial or monocarpic perennial, while the other studied species are perennial. Self-compatibility is most likely to occur in *C. oleraceum* among the studied species (Bureš et al. 2010). First evidence for selection at home vs. away sites in *C. oleraceum* and *C. biennis* suggest that life-history traits are important for a local adaptation at small scale to plant environments of low and high neighbourhood diversity.

Acknowledgements

We acknowledge W.W. Weisser and E.-D. Schulze for coordinating the Jena Experiment. We thank all people helping with the management of the experiment, especially A. Weigelt as field coordinator, the gardeners and many student helpers. We thank two anonymous reviewers for their valuable comments on the manuscript. The Jena Experiment is funded by the German Science Foundation (FOR 456) with support from the Friedrich Schiller University of Jena, the Max Planck Society and the Swiss National Science Foundation (31003A-107531 to B.S.).

Appendix A: Supplementary Material

The online version of this article contains additional supplementary data. Please visit XXXX.

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Table 1: Analyses of variance measured traits combining all species

	Plant height			Relative plant height			Leaf length			Number of leaves			Shoot mass		
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Species (SP)	4	356.44	214.87***	4	5.87	20.10***	4	86.19	69.30***	4	130.77	67.13***	4	516.45	85.98***
Actual environment (A)	1	82.73	250.80***	1	48.86	759.13***	1	122.68	600.60***	1	0.02	0.08	1	11.75	8.62**
Original environment (O)	1	0.02	0.05	1	0.02	0.26	1	0.08	0.25	1	0.92	1.90	1	4.03	2.68
SP x A	4	29.26	22.18***	4	3.63	14.10***	4	10.56	12.92***	4	31.88	31.33***	4	142.99	26.22***
SP x O	4	5.96	3.59*	4	0.27	0.92	4	4.20	3.38*	4	1.73	0.89	4	8.87	1.48
A x O	1	0.09	0.27	1	0.01	0.09	1	<0.01	<0.01	1	0.90	3.53	1	0.11	0.08
SP x A x O	4	3.62	2.74*	4	0.07	0.26	4	1.31	1.61	4	0.99	0.98	4	4.54	0.83
Plot	2	2.37	3.59*	2	0.15	1.17	2	0.65	1.59	2	5.31	10.44***	2	0.22	0.08
Plot of seed family	4	0.29	0.17	4	0.36	1.23	4	0.59	0.48	4	0.42	0.21	4	2.65	0.44
Seed family	36	14.93	1.26	36	2.63	1.13	36	11.19	1.52*	36	17.53	1.91**	36	57.06	1.10
Residuals	663	218.70		663	42.67		662	135.22		662	168.42		655	890.11	

	Average leaf biomass			Inflorescence biomass			Leaf damage			Survival			Proportion reproductive plants		
Source of variation	df	SS	F	df	SS	F	df	SS	F	ndf	SS	F	ndf	SS	F
Species (SP)	4	282.20	73.32***	3	147.69	28.62***	4	305.89	110.66***	4	14.81	3.70*	4	77.58	19.21***
Actual environment (A)	1	28.93	37.58***	1	8.19	9.93***	1	29.23	46.58***	1	7.28	7.28**	1	2.04	2.04
Original environment (O)	1	2.81	2.92	1	0.52	0.30	1	1.42	2.05	1	0.29	0.29	1	0.02	0.02
SP x A	4	32.77	10.64***	2	17.47	10.59***	4	65.43	26.07***	4	13.41	3.35*	4	18.25	4.56***
SP x O	4	5.47	1.42	2	4.02	1.17	4	0.68	0.25	4	5.21	1.30	4	0.02	0.01
A x O	1	0.53	0.69	1	8.24	9.99**	1	3.03	4.83*	1	<0.01	<0.01	1	0.96	0.96
SP x A x O	4	2.67	0.87	1	0.28	0.33	4	3.21	1.28	4	3.58	0.89	4	0.01	<0.01
Plot	2	7.51	4.88**	2	1.54	0.94	2	9.41	7.50***	2	0.22	0.11	2	3.15	1.57
Plot of seed family	4	4.39	1.14	3	0.93	0.18	4	8.11	2.93*	4	2.66	0.66	4	3.98	0.98
Seed family	36	34.64	1.25	22	37.85	2.09**	36	26.26	1.10	vc ± se	0.05	0.04	vc ± se	0.46	0.24
Residuals	654	503.51		166	136.87		663	414.81		md ± se	0.84	0.04	md ± se	0.65	0.04

Listed are degrees of freedom (df) and the sum of squares (SS) for generalized linear models (GLM), numerator degrees of freedom (ndf), Wald statistic (Wald stat.), variance component for seed family with approximate standard error (vc ± se) and residual mean deviance with approximate standard error (md ± se) for Generalized Linear Mixed Models (GLMM) and F ratios (F). Levels of significance are *: $p \leq 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

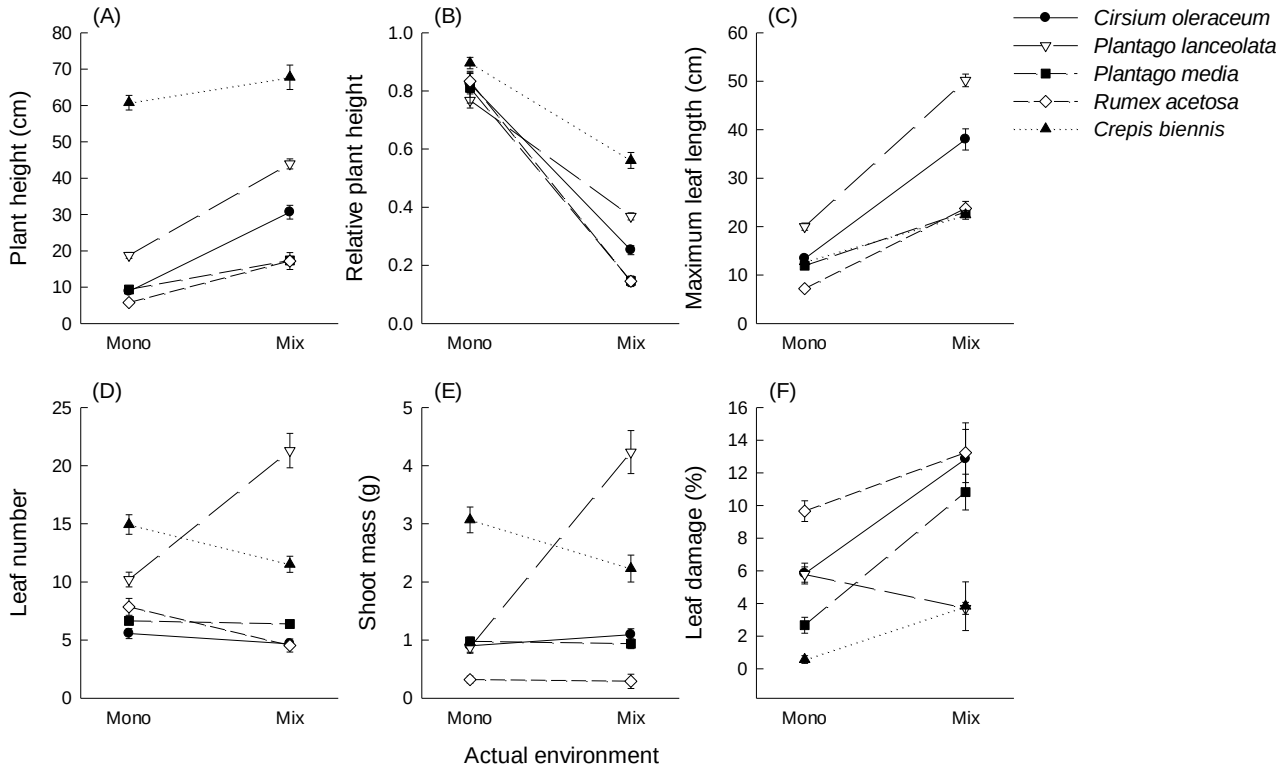

Fig. 1. Plant height (A), relative plant height (B), maximum leaf length (C), leaf number (D), shoot biomass (E) and leaf damage (F) of studied species in response to different actual environments (monoculture vs. 60-species mixtures). Values are per species means (± 1 SE) for all plants per actual environment irrespective of the original environment.

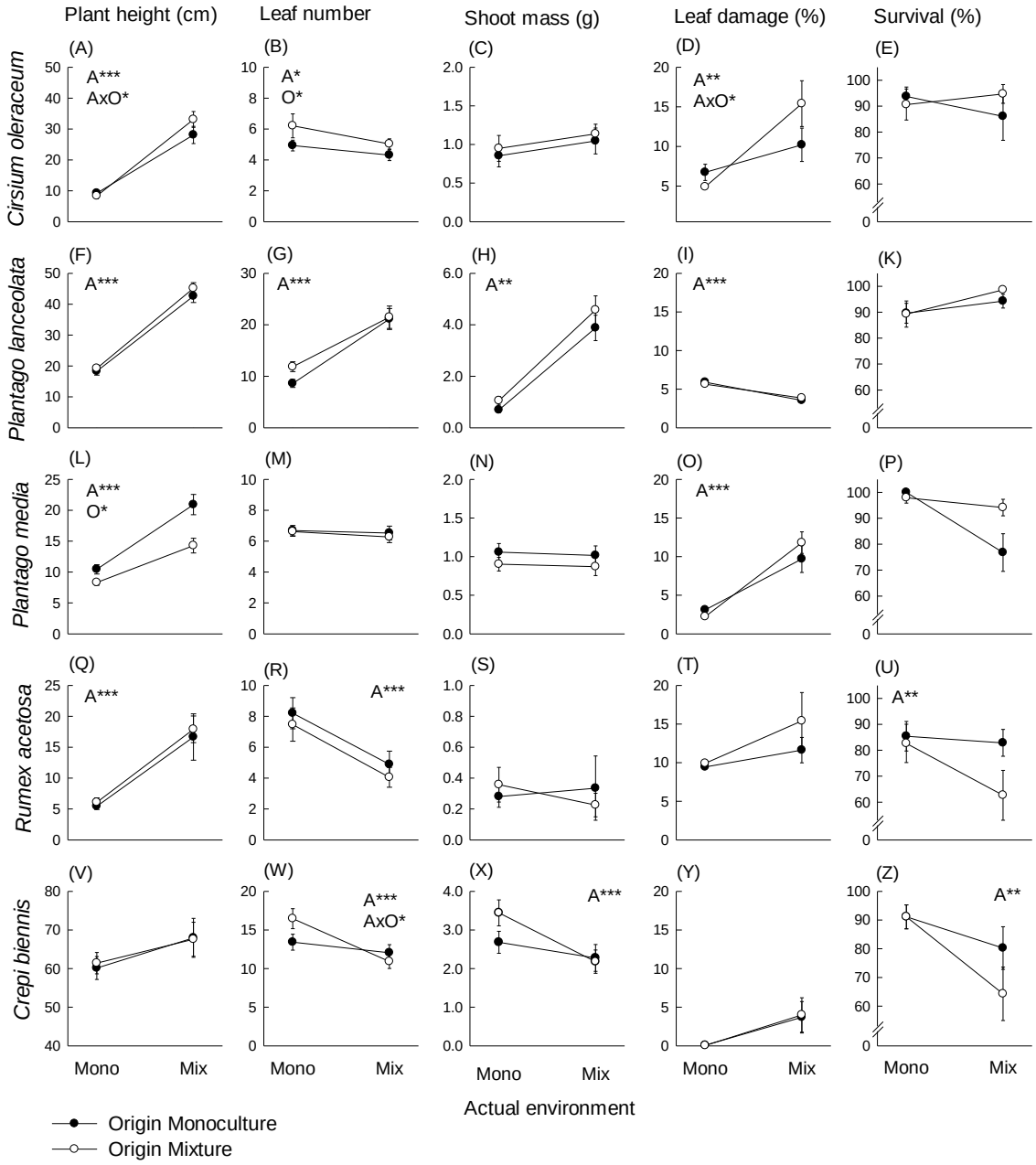

Fig. 2: Reaction norms for *Cirsium oleraceum* (first row, A-E), *Plantago lanceolata* (second row, F-K), *Plantago media* (third row, L-P), *Rumex acetosa* (fourth row, Q-U) and *Crepis biennis* (fifth row, V-Z) differing in the original environment of seed families (monoculture and 60-species mixture) for plant height, leaf number, shoot biomass, leaf damage and survival in response to different actual environments (monocultures and 60-species mixtures). For each species maternal plants grew 5 years in monocultures or mixtures before seeds of different mother individuals, were collected, and seedlings originating from these seed families were replanted or transplanted to their "home" or "away" site, respectively. In cases where original environment (O), actual environment (A) or actual by original environment interactions (A × O) were statistically significant (see Table 2 Appendix A), their significance levels are indicated (*: $p \leq 0.05$, **: $p < 0.01$, and ***: $p < 0.001$). Values are means (± 1 SE) for all plants per actual and original environment. We do not present additional variables (Table 1), where we did not find significant effects of original environment or actual × original environment interactions in any species.

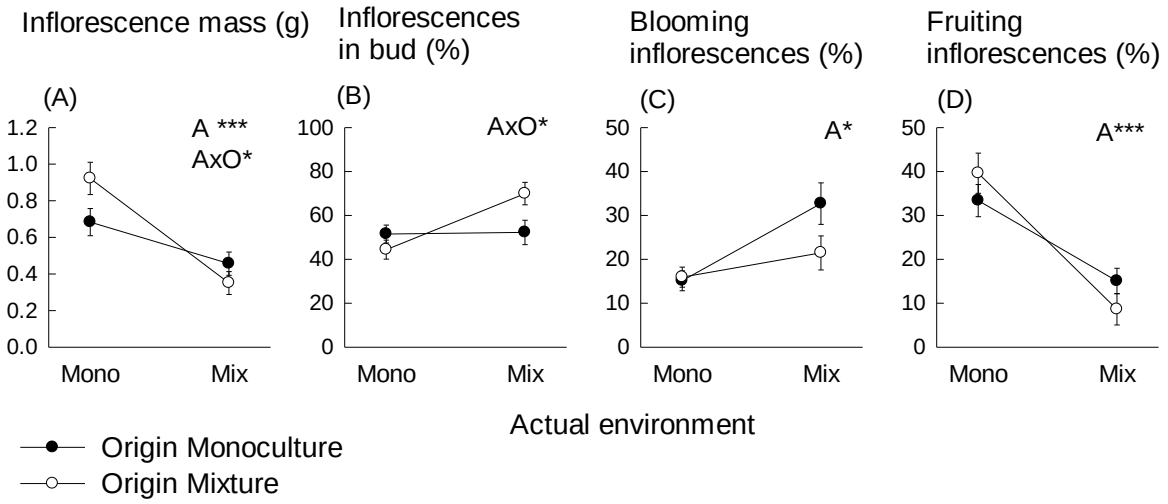

Fig. 3: Reaction norms for *Crepis biennis* differing in the original environment of seed families (monoculture and 60-species mixture) for (A) inflorescence biomass, (B) proportion of bud-forming inflorescences, (C) proportion of flowering inflorescences, and (D) proportion of fruiting inflorescences per plant individual in response to different actual environments (monoculture and 60-species mixture). Maternal plants grew five years in monocultures and mixtures before seeds of different mother individuals, were collected, and seedlings originating from these seed families were replanted or transplanted to their "home" or "away" site, respectively. In cases where actual environment (A) and actual by original environment interactions (A × O) were statistically significant (see Table 3 Appendix A), their significance levels are indicated (*: $p \leq 0.05$, **: $p < 0.01$, and ***: $p < 0.001$). Values are means (± 1 SE) for all plants per actual and original environment.

Appendix

List of plant species used in the design of the Jena Experiment and their assignment to plant functional groups (Roscher et al. 2004).

Tall herbs
Achillea millefolium L. (Asteraceae)
Anthriscus sylvestris (L.) Hoffm. (Apiaceae)
Campanula patula L. (Campanulaceae)
Cardamine pratensis L. (Brassicaceae)
Carum carvi L. (Apiaceae)
Centaurea jacea L. (Asteraceae)
Cirsium oleraceum (L.) Scop. (Asteraceae)
Crepis biennis L. (Asteraceae)
Daucus carota L. (Apiaceae)
Galium album Mill. (Rubiaceae)
Geranium pratense L. (Geraniaceae)
Heracleum sphondylium L. (Apiaceae)
Knautia arvensis (L.) J.M. Coult. (Dipsacaceae)
Leucanthemum vulgare Lam. (Asteraceae)
Pastinaca sativa L. (Apiaceae)
Pimpinella major (L.) Huds. (Apiaceae)
Ranunculus acris L. (Ranunculaceae)
Rumex acetosa L. (Polygonaceae)
Sanguisorba officinalis L. (Rosaceae)
Tragopogon pratensis L. (Asteraceae)

Legumes
Lathyrus pratensis L. (Fabaceae)
Lotus corniculatus L. (Fabaceae)
Medicago lupulina L. (Fabaceae)
Medicago x varia Martyn (Fabaceae)
Onobrychis viciifolia Scop. (Fabaceae)
Trifolium campestre Schreb. (Fabaceae)
Trifolium dubium Sibth. (Fabaceae)
Trifolium fragiferum L. (Fabaceae)
Trifolium hybridum L. (Fabaceae)
Trifolium pratense L. (Fabaceae)
Trifolium repens L. (Fabaceae)
Vicia cracca L. (Fabaceae)

Small herbs
Ajuga reptans L. (Lamiaceae)
Bellis perennis L. (Asteraceae)
Glechoma hederacea L. (Lamiaceae)
Leontodon autumnalis L. (Asteraceae)
Leontodon hispidus L. (Asteraceae)
Plantago lanceolata L. (Plantaginaceae)
Plantago media L. (Plantaginaceae)
Primula veris L. (Primulaceae)
Prunella vulgaris L. (Lamiaceae)
Ranunculus repens L. (Ranunculaceae)
Taraxacum officinale Wiggers (Asteraceae)
Veronica chamaedrys L. (Scrophulariaceae)

Grasses
Alopecurus pratensis L. (Poaceae)
Anthoxanthum odoratum L. (Poaceae)
Arrhenatherum elatius (L.) Presl (Poaceae)
Avenula pubescens (Huds.) Dum. (Poaceae)
Bromus erectus Huds. (Poaceae)
Bromus hordeaceus L. (Poaceae)
Cynosurus cristatus L. (Poaceae)
Dactylis glomerata L. (Poaceae)
Festuca pratensis Huds. (Poaceae)
Festuca rubra L. (Poaceae)
Holcus lanatus L. (Poaceae)
Luzula campestris (L.) Dc. (Juncaceae)
Phleum pratense L. (Poaceae)
Poa pratensis L. (Poaceae)
Poa trivialis L. (Poaceae)
Trisetum flavescens (L.) P. Beauv. (Poaceae)

Table 2: Analyses of variance of measured traits for each species separately

	Cirsium oleraceum			*Plantago lanceolata*			*Plantago media*			*Rumex acetosa*			*Crepis biennis*		
Plant height															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	41.24	89.91***	1	34.65	165.96***	1	11.34	41.92***	1	24.74	49.76***	1	0.02	0.05
Original environment (O)	1	0.20	0.39	1	0.43	1.22	1	4.80	8.31*	1	0.45	1.15	1	0.12	0.45
A x O	1	2.86	6.23*	1	0.01	0.04	1	0.81	2.98	1	0.03	0.07	1	<0.01	0.01
Plot	2	2.57	2.80	2	0.31	0.75	2	3.21	5.93**	2	0.63	0.63	2	0.22	0.35
Plot of seed family	3	1.19	0.77	1	0.03	0.08	2	0.35	0.30	1	0.40	1.02	1	0.08	0.29
Seed family	3	1.56	1.13	9	3.15	1.68	8	4.62	2.13*	5	1.96	0.79	7	1.83	0.83
Residuals	106	48.62		160	33.40		162	43.84		91	45.24		3	43.09	
Relative plant height															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	9.67	225.00***	1	7.01	203.26***	1	19.55	141.13***	1	12.09	265.96***	1	4.17	97.74***
Original environment (O)	1	<0.01	0.01	1	0.06	0.90	1	0.23	1.44	1	<0.01	<0.01	1	<0.01	<0.01
A x O	1	0.05	1.15	1	0.01	0.41	1	0.01	0.04	1	<0.01	0.07	1	<0.01	0.01
Plot	2	0.11	1.31	2	0.10	1.39	2	0.08	0.28	2	0.05	0.55	2	<0.01	0.02
Plot of seed family	3	0.09	0.44	1	<0.01	0.07	2	0.31	0.98	1	<0.01	0.02	1	<0.01	0.04
Seed family	3	0.22	1.69	9	0.60	1.92	8	1.26	1.13	5	0.23	0.99	7	0.31	1.03
Residuals	106	4.56		160	5.52		162	22.45		91	4.14		3	5.80	
Leaf length															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	28.96	88.50***	1	39.31	321.53***	1	14.14	86.99***	1	38.07	111.61***	1	12.75	86.78***
Original environment (O)	1	0.49	2.08	1	0.26	0.91	1	2.60	7.62*	1	0.46	1.70	1	0.48	1.91
A x O	1	0.85	2.59	1	<0.01	0.02	1	0.40	2.44	1	0.06	0.18	1	0.00	0.02
Plot	2	1.49	2.27	2	0.11	0.44	2	2.36	7.26***	2	0.42	0.61	2	0.49	1.66
Plot of seed family	3	0.98	1.39	1	<0.01	<0.01	2	0.59	0.87	1	0.65	2.40	1	<0.01	<0.01
Seed family	3	0.71	0.72	9	2.58	2.35*	8	2.73	2.10*	5	1.35	0.79	7	1.75	1.70
Residuals	106	34.69		160	19.56		162	26.34		91	31.04		3	19.83	
Leaf number															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	0.75	4.81*	1	20.49	55.90***	1	0.24	1.99	1	8.18	19.74***	1	2.43	10.98**
Original environment (O)	1	0.91	13.32*	1	1.15	1.72	1	0.02	0.08	1	0.21	0.25	1	0.18	0.95
A x O	1	0.01	0.05	1	0.92	2.50	1	<0.01	0.03	1	0.01	0.02	1	0.96	4.32*
Plot	2	0.34	1.07	2	2.49	3.40	2	2.25	9.46***	2	6.35	7.66***	2	1.33	3.01
Plot of seed family	3	0.93	4.53	1	0.31	0.47	2	0.01	0.02	1	1.67	1.96	1	0.15	0.82

	Cirsium oleraceum			*Plantago lanceolata*			*Plantago media*			*Rumex acetosa*			*Crepis biennis*		
Seed family	3	0.20	0.44	9	6.03	1.83	8	1.99	2.10	5	4.26	2.06	7	1.31	0.84
Residuals	106	16.55		160	58.63		161	19.11		91	37.69		136	30.08	
Shoot mass															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	0.92	0.58	1	140.32	94.31**	1	3.02	3.11	1	3.30	1.77	1	7.73	7.33**
Original environment (O)	1	2.35	3.52	1	5.99	2.86	1	1.90	1.36	1	0.02	0.01	1	2.10	1.92
A x O	1	2.86	1.80	1	0.79	0.53	1	0.27	0.27	1	0.03	0.01	1	0.71	0.67
Plot	2	7.00	2.21	2	4.54	1.52	2	4.08	2.10	2	6.64	1.78	2	1.74	0.83
Plot of seed family	3	4.94	2.47	1	0.00	0.00	2	0.79	0.28	1	4.90	2.70	1	0.33	0.30
Seed family	3	2.00	0.42	9	18.85	1.41	8	11.20	1.44	5	9.08	0.97	7	7.67	1.04
Residuals	106	168.11		158	235.07		159	154.81		89	165.87		135	142.39	
Average leaf mass															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	3.34	2.91	1	50.63	65.12***	1	0.59	0.96	1	1.08	0.96	1	6.00	15.35***
Original environment (O)	1	0.34	0.63	1	2.24	2.31	1	1.11	1.34	1	0.90	0.64	1	3.77	4.40
A x O	1	2.56	2.23	1	<0.01	<0.01	1	0.29	0.46	1	0.24	0.21	1	0.11	0.28
Plot	2	6.18	2.69	2	2.43	1.56	2	6.18	4.97**	2	0.75	0.34	2	0.31	0.40
Plot of seed family	3	6.35	3.99	1	0.47	0.49	2	0.60	0.36	1	0.98	0.69	1	0.35	0.41
Seed family	3	1.59	0.46	9	8.71	1.25	8	6.58	1.32	5	7.05	1.26	7	6.00	2.19*
Residuals	106	121.89		158	122.83		158	98.27		89	99.75		135	52.78	
Leaf damage															
Source of variation	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Actual environment (A)	1	6.79	9.75**	1	5.20	12.54***	1	79.65	106.86***	1	1.07	2.18	1	2.15	3.23
Original environment (O)	1	0.49	0.73	1	0.11	0.18	1	0.34	0.75	1	0.96	0.90	1	0.00	0.00
A x O	1	3.58	5.13*	1	0.02	0.04	1	2.32	3.11	1	0.05	0.10	1	0.28	0.42
Plot	2	4.94	3.54*	2	2.26	2.73	2	17.56	11.78***	2	0.52	0.53	2	3.47	2.61
Plot of seed family	3	1.44	0.71	1	0.08	0.12	2	7.66	8.58**	1	0.18	0.17	1	2.25	2.48
Seed family	3	2.03	0.97	9	5.58	1.50	8	4.46	0.60	5	5.33	2.18	7	6.35	1.36
Residuals	106	73.86		160	66.30		162	119.27		91	44.50		136	90.58	
Survival															
Source of variation	df	Wald stat	Fpr	df	Wald stat	Fpr	df	Wald stat	Fpr	df	Wald stat	Fpr	df	Wald stat	Fpr
Actual environment (A)	1	0.01	0.01	1	0.30	0.30	1	3.27	3.27	1	4.03*	4.03	1	8.01	8.01**
Original environment (O)	1	0.05	0.05	1	0.34	0.34	1	1.36	1.36	1	1.30	1.30	1	0.37	0.37
A x O	1	0.30	0.30	1	1.31	1.31	1	0.00	0.00	1	0.77	0.77	1	0.47	0.47
Plot	2	0.69	0.35	2	3.14	1.57	2	1.93	0.96	2	0.30	0.15	2	2.98	1.49
Plot of seed family	3	1.61	0.48	1	0.76	0.76	2	1.51	0.76	1	0.04	0.04	1	0.23	0.23

Table 2: Analyses of variance of measured traits for each species separately

	Cirsium oleraceum			*Plantago lanceolata*			*Plantago media*			*Rumex acetosa*			*Crepis biennis*		
Seed family	vc ± se	0.2	0.26	vc ± se	0.11	0.11	vc ± se	0.34	0.25	vc ± se	1.05	---	vc ± se	0.05	0.08
Residuals	md ± se	0.74	0.1	md ± se	0.75	0.09	md ± se	0.53	0.06	md ± se	0.00	0.13	md ± se	0.96	0.11

Listed are degrees of freedom (df) and the sum of squares (SS) for generalized linear models (GLM), numerator degrees of freedom (ndf) and Wald statistic (Wald stat.), variance component for seed family with approximate standard error (vc ± se) and residual mean deviance with approximate standard error (md ± se) for Generalized Linear Mixed Models (GLMM) and F ratios (F). Levels of significance are *: $p \leq 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Table 3: Analyses of variance of measured reproductive traits (inflorescence biomass, proportion of reproductive plants, proportion of buds per plant individual, proportion of blooming inflorescences per plant individual and proportion of fruiting inflorescences per plant individual) of *Crepis biennis*.

	Inflorescence biomass			**Proportion reproductive plants**			**Proportion bud**			**Proportion blooming inflorescences**			**Proportion fruiting inflorescences**		
Source of variation	df	SS	F	ndf	Wald stat.	Fpr	ndf	Wald stat.	Fpr	ndf	Wald stat.	Fpr	ndf	Wald stat.	Fpr
Actual environment (A)	1	21.23	28.18	1	4.82	4.82	1	1.48	1.48	1	16.08	16.08	1	12.23	12.23
Original environment (O)	1	0.24	0.3	1	0.07	0.07	1	0.01	0.01	1	0.15	0.15	1	0.21	0.21
A x O	1	4.88	6.47	1	0.19	0.19	1	2.42	2.42	1	2.42	2.42	1	3.05	3.05
Plot	2	0.81	0.53	2	1.01	0.51	2	0.55	0.28	2	0.55	0.28	2	1.37	0.68
Plot of seed family	1	0.02	0.03	1	1.13	1.13	1	2.73	2.73	1	2.73	2.73	1	0.29	0.29
Seed family	7	5.42	1.03	vc ± se	0.00	---	vc ± se	0.09	0.09	vc ± se	0.09	0.09	vc ± se	0.09	0.11
Residuals	117	88.17		md ± se	1.10		md ± se	2.77	0.37	md ± se	2.77	0.37	md ± se	5.16	0.68

Listed are degrees of freedom (df) and the sum of squares (SS) for generalized linear models (GLM), numerator degrees of freedom (ndf) and Wald statistic (Wald stat.) for Generalized Linear Mixed Models (GLMM) and F ratios (F). Levels of significance are *: $p \leq 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Chapter 3

Rapid r- / K-selection in *Taraxacum officinale* in response to density-independent mortality / increasing plant diversity

Annett Lipowsky, Christiane Roscher, Jens Schumacher & Bernhard Schmid (Manuscript)

Abstract

Background: Differential selection between clones of apomictic species may result in ecological differentiation without mutation and recombination, thus offering a simple system to study adaptation and life-history evolution in plants.

Methodology/Principal Findings: We caused density-independent mortality by weeding to colonizer (= non-sown) populations of the largely apomictic *Taraxacum officinale* (Asteraceae) over a 5-year period in grassland communities of different sown plant diversity (Jena Experiment). We compared the offspring of these colonizer populations with resident populations deliberately sown into similar communities at the beginning of the experiment. Plants raised from cuttings and seeds of colonizer and resident populations were grown under uniform conditions in a common garden for one year. We found that offspring from colonizer populations had higher reproductive output with a higher number of flower heads and seeds per plant, which was in general agreement with predictions of r-selection theory and classical results from comparative studies in natural populations. Offspring from resident populations had higher root biomass, fewer flower heads and higher individual seed mass as predicted under K-selection. More diverse communities appeared to exert stronger K-selection on resident populations resulting in larger shoot and reproductive biomass, increased seed mass per flower head and larger flower head diameter of their offspring.

Conclusions/Significance: The differentiation into r- and K-strategists suggests that clones with characteristics of r-strategists were competitively excluded from resident populations growing in grasslands of increasing plant diversity over a 5-year period, while r-selected clones could spread in the weeded plots through rapid colonization. Our results show that different selection pressures through density-independent mortality or plant competition may result in a rapid genetic differentiation within a largely apomictic species such as *T. officinale*.

Introduction

The evolutionary potential of populations or species is largely determined by the amount and patterns of genetic variation. Natural selection may shape the adaptation of populations in response to local environmental conditions such as disturbance or competition, but little is known about the time-scale of genetic divergence. *Taraxacum officinale* (common dandelion) is a species aggregate with variable ploidy level, mating system and degree of reproductive isolation. However, populations even in strictly apomictic regions can be genetically highly

variable [1]. Several studies have shown that different clones in such populations are ecologically differentiated [2,3]. In a citation classic, Gadgil and Solbrig [2] studied the offspring of four clones or biotypes of *T. officinale* originating from natural successional habitats with different levels of disturbance through trampling and mowing in competition experiments and showed that plants from intensively disturbed habitats had traits expected under r-selection. According to the biogeographic theory of MacArthur and Wilson [4,5], populations living in disturbed habitats spend most of their time in exponential growth and should therefore develop traits that allow them to rapidly colonize a habitat and have high intrinsic rates of growth. In other words, they should become r-strategists which are supposed to be particularly successful in unstable environments with density-independent mortality [2,6] and fluctuating resource availability [7]. In contrast, populations spending most of their time in stationary phase should develop traits that allow them to persist and compete against con-specifics and other species, having high asymptotic population size, hence being K-strategists [5]. A high competitiveness for limiting resources is a prerequisite to stay permanently established in such relatively stable environments with density-dependent mortality and relatively constant resource availability [7]. Because trade-offs may exist between the two strategies, it is expected that no species could be both r- and K-strategist at the same time [2,6]. Thus, r-strategists may maximize their reproduction and dispersal efforts only at the cost of reduced competitiveness, whereas K-strategist may invest more resources into vegetative biomass and persistence at the cost of reduced reproductive effort [8].

To test experimentally if r- / K-selection can occur in a species, a first prerequisite is that the species should be genetically polymorphic with regard to the relevant traits under selection. Genetic differentiation within and between plant populations in response to the biotic and abiotic environment is common [9,10]. Selection pressure on competitive traits should favour the establishment of well-adapted genotypes in relevant timescales [11]. Genotype-specific responses to disturbance and competition have been reported repeatedly from studies in natural systems as well as in experimental systems combining different species [12-15]. The strongest K-selection may be expected in species-rich habitats with constantly high competition, even if this is predominantly inter- rather than intra-specific. However, effects of species richness as a selection pressure on plant life-history traits and genetic differentiation within populations are poorly understood [16-20].

The particular traits that enable plants to live in disturbed habitats with high density-independent mortality (r-strategy) are similar to those of invading species, e.g. short generation time, large seed number, small seed size and rapid growth [21-23]. Traits that

enable plants to live in less variable environments with density-dependent mortality (K-strategy) are those of good survivors and competitors, e.g. high investment in root biomass and individual seed mass [24,25].

Here we revisited the question about r- and K-selection in *T. officinale* using a grassland biodiversity experiment in Jena, Germany [26]. Gadgil and Solbrig [2] compared in their study plants obtained from different habitats, which presumably differed in density-independent mortality regimes. In contrast to their approach, we deliberately created r- and K-selection regimes ourselves. Our controlled r-selection regime was imposed for a period of 5 years by weeding *T. officinale* (causing density-independent mortality) in plots of varying species richness, where it did not belong to the sown species combinations and was considered as an invader. We compared this treatment with a K-selection regime, in which *T. officinale* was sown as a resident species in the plots of increasing plant species richness and was not weeded during the 5 years. After the 5 years of selection, we grew offspring from seeds and cuttings of both types of plants under uniform conditions in a common garden. We hypothesized that 5 years of differential experimental selection resulted in a genetic differentiation among dandelion populations. If this would happen, it could be due to differential selection among co-occurring clones because *T. officinale* is largely apomictic. An alternative possibility would be differential maternal effects which could be larger in cutting- than in seed-derived material. The offspring of r-selected *T. officinale* should show the traits expected for r-strategists, in particular increased reproductive output, higher shoot:root ratio and fast reproduction, and the offspring of K-selected *T. officinale* should show traits expected for K-strategists, in particular increased allocation to vegetative growth and increased plant and propagule size. In addition, because we assumed that increasing inter-specific competition in plant communities of higher species richness exerts stronger K-selection than do species-poor communities, we hypothesized that these traits expected for K-strategists should be positively selected with increasing species richness of plots from which K-selected offspring were collected.

Results

DIFFERENCES BETWEEN CUTTINGS AND PLANTS GROWN FROM SEEDS

Plants raised from cuttings differed from plants raised from seeds in a number of vegetative (e.g. shoot biomass, vegetative biomass, leaf length) and reproductive traits (e.g. average seed mass; Table 1). Cuttings were generally lighter, produced shorter leaves and had a lower

mean seed mass and a lower seed mass per flower head and per plant than plants grown from seeds (Table 2).

DIFFERENCES BETWEEN k- AND R-SELECTED PLANTS

Standard germination tests with seeds revealed considerable variation in germination rates among maternal plants. Seeds of 14% of maternal plants either failed to germinate completely or had germination rates below 10%; seeds of 28% of maternal plants had germination rates above 70%. Average germination rates of seeds from K-selected (i.e. resident) and r-selected (i.e. weedy, colonizing) maternal plants did not differ significantly (Fig. 1, Table 1).

Combining cuttings and seedlings, shoot biomass did not differ significantly between r- and K-selected plants (Fig. 2a, Table 1), but r-selected plants had lower root biomass than K-selected plants and consequently higher shoot:root ratios (Fig. 2b, c, Table 1). Aboveground vegetative and reproductive biomass was not significantly different between r- and K-selected plants (Fig. 2d, e, Table 1), but r-selected plants produced more leaves (of similar length) than K-selected plants (Fig. 2f, g). In total, 84% of all cuttings and 75% of all plants grown from seeds produced flowers at harvest time. K-selected plants produced fewer flower heads than did r-selected plants (Fig. 3a, Table 1) and were less likely to flower (74% of K-selected vs. 82% of r-selected plants). Flower-head diameter was not significantly different between r- and K-selected plants (Fig. 3b, Table 1). However, r-selected plants had a lower seed mass per flower head (Fig. 3c, Table 1). Nevertheless, due to smaller average seed mass (Fig. 3d, Table 1) and the higher number of flower heads, r-selected plants produced more seeds per plant and had a higher total seed mass per plant (Fig. 3e-f, Table 1).

Significant interaction terms between plant origin and the selection regime ("S vs. C × rK") suggested that effects of the selection regime on reproductive traits differed to some degree between cuttings and seedlings. Mean seed mass was reduced and seed number per plant was strongly increased in cuttings of r-selected plants (Fig. 4a, b, Table 1). In contrast, mean seed mass did not vary between r- and K-selected plants and seed number per plant was only little increased in r-selected plants, when plants were grown from seeds. Effects of the selection regime on other reproductive traits (i.e. number of flower heads, seed mass per flower head, seed mass per plant) did not differ between cuttings and plants grown from seeds (Table 1). Finally, r-selected plants also showed more precocity, flowered on average 4.1 days earlier and produced mature seeds 1.5 days earlier than K-selected plants (Fig. 3g, h, Table 1). Again the selection regime had stronger effects on cuttings. Seed maturity of r-selected plants was noticeably more advanced in cuttings, while differences between r- and K-selected plants

were less pronounced in plants grown from seeds (Fig. 4c). On average, the height of the flower stalk at the time of seed maturity did not differ significantly between r- and K-selected plants (Fig. 3h, Table 1).

SELECTION RESPONSE TO BIODIVERSITY

High species richness selected for *T. officinale* plants of large size (large shoot and reproductive biomass, large flower heads, high seed mass per flower head; Fig. 2a, e and Fig. 3b, c, Table 1). Average germination rates of seed material collected in the biodiversity experiment increased with increasing species richness (Fig. 1, Table 1). Reproductive biomass, seed mass per plant and number of flowerheads showed a stronger selection response to biodiversity in r-selected plants (significant interaction term "rK × log SR", Table 1) although the measured values are higher in K selected plants. Selection effects of species richness on the studied characteristics of *T. officinale* did only differ between cuttings and plants grown from seeds in the height of flowering stalk (significant interaction terms "S vs. C × log SR", Fig. 3i, Table 1).

Discussion

In the present study we wanted to assess whether 5 years of density-independent mortality due to regular weeding and density-dependent mortality caused by competitive interactions in plant communities of different species richness can cause genetic differentiation between colonizer and resident populations of *T. officinale*. Based on a common garden experiment with offspring raised from cuttings or seeds of these populations, we could show for a number of traits characterizing biomass allocation and reproductive output that density-independent mortality (r-selection) caused different adaptive responses than density-dependent mortality (K-selection). We assume that colonizer as well as resident populations represent a mixture including genotypes from the originally sown seed material (probably the major component of the mixture) and invading genotypes from the surroundings of the experimental site (probably the minor component of the mixture).

One possibility to explain our results could be that resident populations were more or less exclusively derived from sown seeds *and* that these seeds originated from already K-selected populations whereas the colonizer populations were more or less exclusively derived from seeds dispersing from local populations *and* that these were already r-selected. However, this explanation is unlikely because the seeds for the establishment of the biodiversity experiment were collected in natural habitats of different successional age and with diverse regimes of

disturbance and competition (fallow land, established grasslands). Similarly, seed sources in the near surroundings of the Jena experimental site comprise established grasslands, agricultural land and ruderal sites. A high clonal diversity with few clones widespread and many clones restricted to single populations is typical for the genetic population structure of *T. officinale* [1,27]. Nevertheless, even if a "pre-adaptation of K-strategists" in resident populations and "pre-adaptation of r-strategists" in colonizer populations would explain our results, it would still show that 5 years of deliberate r- and K-selection kept the original populations apart. Additionally, the effects of species richness on the degree of K-selection among resident populations is even less likely to be due to such biased mixing of differently but "correctly pre-adapted" seed populations. However, we do not know to which extent the heritable variation observed between r- and K-selected plants was potentially due to maternal effects or epigenetic inheritance [28,29].

DIFFERENCES BETWEEN CUTTINGS AND PLANTS GROWN FROM SEEDS

An indication for a role of maternal effects would have been a stronger selection response in plants derived from cuttings as compared to plants derived from seeds [30]. In our study, differences between cuttings and plants grown from seeds were found in vegetative biomass, maximum leaf length and seed output (seed mass per flower head and plant, average seed mass). This variation was presumably due to different starting capital and different soil substrate provided to cuttings and seed-derived plants. Cuttings and offspring from seeds did not differ in traits assumed to be less influenced by starting capital and soil nutrients, e.g. the number of leaves and the number of flower heads. Nevertheless, there was some indication that the selection regime caused by density-independent mortality by weeding had more pronounced effects on cuttings, i.e. reduction in mean seed mass, increase in seed number per plants and precocity in seed maturity was stronger in r-selected plants grown from cuttings compared to those cultivated from seeds (Fig. 4). This suggests that adaptive maternal effects occurred in cuttings or that seeds were not fully apomictic and thus non-adaptive variation was recreated by genetic recombination in seeds of maternal plants.

r-AND K-SELECTION IN TARAXACUM OFFICINALE

In contrast to a study by Molgaard [31], not only morphological plasticity in response to different disturbance regimes (mowing height), but also heritable, presumably genetic differentiation between the differently selected *T. officinale* populations was found in our experiment. The results of our study show that the concept of r- and K-strategy [2,5,6] is

applicable to the strategies developed in populations of *T. officinale* in response to different selection regimes over a 5-year period. These two opposite strategies [2,21] seem to be adapted in one extreme to unstable environments where resource fluctuations and density-independent mortality are major selection pressures (r-selection) and in the other extreme to stable environments where habitat carrying capacity, competitive interactions and density-dependent mortality are major selection pressures (K-selection). Natural selection should drive a trade-off between both strategies and maximize either the success of r- or K-strategists under these contrasting selection regimes.

In our common garden experiment, plants originating from repeatedly weeded colonizer populations showed the expected characteristics of r-strategists. These plants, compared with resident plants, had larger shoot:root ratios and invested a greater proportion of their available resources into a maximization of reproductive output, i.e. they produced a higher number of flower heads and a larger number of seeds. At the same time seed mass of these plants was lower, presumably enhancing wind-dispersal [32] and thus increasing colonization potential [33]. All these features, including faster reproduction, are reported as common life-history traits not only of r-strategists, but also be typical characteristics of weedy or invasive species [34,35].

In contrast, plants originating from resident populations needing more time for flowering and seed maturation and producing larger seeds with higher germination rates fit the profile expected for K-selected species [6]. Heavier achenes are more likely to germinate successfully in *T. officinale* [36] and correlate positively with post-germination performance such as seedling mass [37]. The resident populations also allocated a greater proportion of their biomass to roots; and a lower shoot:root ratio is frequently correlated with a higher plant tolerance to competition, drought or frost [38,39]. This feature is not only reported as a typical life-history trait of K-strategists, but also included in concepts developed for differences between early vs. late-successional species, where the latter are also expected to experience intense competition [40]. However, because the applied selection pressures of density-independent mortality vs. competition are explicitly specified as the underlying mechanisms of r- vs. K-selection, we prefer to put our study into this perspective. *Taraxacum officinale* subjected for 5 years to r-selection showed a shortened time to reproduction and had a higher reproductive output, i.e. the plants were optimized for rapid population growth. The same species subjected for 5 years to K-selection invested more biomass into roots and increased individual seed mass.

HIGH SPECIES RICHNESS ENHANCES K-SELECTION

We observed that resident populations after 5 years showed significant heritable differentiation of K-strategy traits in response to the species richness gradient present in the biodiversity field experiment. This indicates that diverse experimental communities imposed stronger K-selection on the residents than did less diverse communities. It is likely that the more diverse experimental communities with higher plant densities and community biomass [41] have exerted a stronger competitive pressure on the *T. officinale* plants. That the colonizer populations did not show this response to increasing community diversity (see Fig. 3) is consistent with the fact that they were r-selected by the density-independent weeding and thus did not have a chance to experience the increased density-dependent competition along the diversity gradient for long enough.

Conclusions

In this study we have investigated whether a short period of 5 years experimentally controlled selection may result in a heritable genetic differentiation among populations of the largely apomictic species *T. officinale*. More than 35 years ago Gadgil and Solbrig [2] reported in a classic comparative study that plants of *T. officinale* originating from sites with presumably different selection regimes over an unknown period had developed traits expected under r- or K-selection. In our experimental approach we created the r- and K-selection regimes ourselves by weeding (density-independent mortality, r-selection) and increasing inter-specific competition along a species richness gradient (K-selection). After 5 years, these selection regimes had resulted in plants that showed the corresponding traits expected under r- and K-selection in a common environment. Although the question about the mechanism behind the genetic differentiation over a short time period is beyond the scope of our study and we found some incidence for maternal effects, it is probable that the selection response was mainly due to differential mortality and asexual reproduction of different clones; recombination or mutation events were probably of minor importance [1]. In this respect *T. officinale* represents a simple system to study adaptive evolution of life-history traits in plants. A similar selection experiment has recently been carried out between inbred lines of the model plant *Arabidopsis thaliana* [42], but we did not find further studies done with wild plants.

Materials and Methods

STUDY SPECIES

Taraxacum officinale Wiggers (Asteraceae) is a herbaceous perennial plant species commonly found in pastures, lawns, along roadsides and in ruderal places. The vegetative plant is characterized by a leaf rosette with a taproot. The species flowers mainly in spring (late April to May). The genus *Taraxacum* contains a complex of diploid sexual and polyploid apomictic forms. Although the plants produce conspicuous flower heads with a copious amount of nectar and pollen, seeds are often produced asexually by diplospory [43]. The seeds disperse through wind or adhesion, often near to the parent plant [44]. In the study region, *T. officinale* is an apomictic, triploid species with almost exclusively asexual reproduction. Numerous agamospecies of *T. officinale* commonly coexist within communities [45-47].

The species has a near cosmopolitan distribution. *Taraxacum officinale* is an invasive (naturalized) weed across North America [48] and Japan [49]; no sexual reproduction was discovered there [9,49,50]. The morphological variability often observed in populations of *T. officinale* has both a genetic [9,14,51] and a plastic [31] component. Despite their predominant asexual reproduction, some single maternal plants have been observed to generate heritable variation among their offspring [44,52].

SELECTION EXPERIMENT

This study was implemented as a part of the Jena Experiment, a large integrated biodiversity experiment in Germany [26]. The experimental site is located in the floodplain of the river Saale near the city of Jena (50°55’ N, 11°35’ E, 130 m a.s.l.). The area around Jena has a mean annual air temperature of 9.3°C and mean annual precipitation is 587 mm [53]. In total, the Jena Experiment has 78 plots of 20 × 20 m size that cover a gradient in species richness (1, 2, 4, 8 to 16) and functional group richness (1–4; legumes, grasses, tall herbs, small herbs). The different mixtures were determined by random drawings with replacement from a pool of 60 species typical of species-rich, semi-natural grasslands (for details see [26]). All species were also grown in monoculture on plots of 3.5 × 3.5 m size. Because of a gradient in soil characteristics across the experimental site all plots were grouped into four blocks parallel to the river. The full experiment was established by sowing in spring 2002.

Seeds were purchased from a commercial supplier (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). Seeds of *T. officinale* used to establish the biodiversity experiment were a mixture collected from natural populations in established grasslands and fallow land in Southern Germany (pers. comm. E. Rieger) and were therefore likely to represent a large

number of genotypes adapted to different environments. *Taraxacum officinale* was included in the experimental species pool and belonged to the community of originally sown (= resident) species in 12 out of 78 large plots and 1 small monoculture. In addition, this species disseminated spontaneously from within and around the experimental plots and colonized all other plots where it had not been sown ([54], Table S1). In these plots, all plants of *T. officinale* and other unwanted species were removed twice every year in April and July. Although the weedy plants of *T. officinale* were pulled out with their roots or cut below the leaf rosette in case they could not be pulled out with roots, they could continually re-establish after the density-independent mortality caused by the weeding, probably by both re-grow from root stumps and re-colonization from seeds. All experimental plots were mown twice a year (early June, September) as usual for extensive hay meadows in the region and did not receive any fertilizer.

EXPERIMENT TO TEST SELECTION RESPONSE

SEEDLINGS

Seeds of randomly chosen maternal plants of *T. officinale* were collected in all 78 large plots of the biodiversity experiment and in a small plot with a *T. officinale* monoculture in May 2007. The seeds of 20 maternal plants were collected in every plot where *T. officinale* belonged to the originally sown species (= 13 resident populations, Table S1). Although some of these plants may have colonized later via seeds from outside, we treat them all as resident plants that we assume to have experienced a K-selection regime (high competition, low density-independent mortality; [2,6]). The seeds of 10 maternal plants were collected in every remaining large plot where *T. officinale* occurred as a weed (= 66 colonizer populations, Table S1). These plants were all considered as weedy plants that experienced an r-selection regime (high density-independent mortality).

Seeds were stored at 4°C until 30 seeds per individual were plated for germination in Petri dishes on moistened filter paper in an unheated glasshouse in early September 2007. Seedlings were counted fortnightly after sowing to determine germination rates. Three seedlings per maternal plant were transplanted into QuickPot™ trays (cylindrical pots with a volume of 0.2 L, Herku-Plast, Germany) filled with compost (GEMES, Germany). Plants were grown in an unheated glasshouse without artificial light. In mid-October plantlets from the QuickPot™ trays were transplanted into 1-L flower pots filled with compost (GEMES, Germany). Pots were buried in a garden bed and covered with garden fleece to avoid frost damage in winter. Plants of one experimental block (block 4) were destroyed by mice during

this period. From mid-February 2008 onwards all remaining plants were cultivated in two glasshouse chambers blocked disaccording to the experimental blocks of the field experiment. Temperatures ranged from 10–15°C during the day and ≥ 5°C at night. Additional light was provided by high-pressure potassium lamps (SON-T AGRO 400 W, Philipps GmbH, Germany) 3 h per day to initiate flower formation. Temperature was increased to 15–22°C at day and 5–10°C at night on 17 March 2008 to simulate spring.

CUTTINGS

In March 2007, *T. officinale* cuttings were taken in all large plots of the biodiversity experiment and a small monoculture. All cuttings had between 2–5 leaves and a height of 1–5 cm. Cuttings with a 5 cm long root were made with a blade. Again, we collected 20 plants from the 13 plots where *T. officinale* was a resident species including the small monoculture, and 10 plants from the 66 plots where *T. officinale* was a weed.

Cuttings were planted into QuickPot™ trays filled with a mixture of quartz sand and soil from the experimental site (1:1) and placed outside the glasshouse on a vegetation-free area. In April 2007, the cuttings were planted into 1-L flower pots filled with the same soil mixture. The plants were fertilized biweekly with 200 ml of a 0.2% compound fertilizer (8:8:6 N:P:K, Wuxal Super, Monheim, Germany) to avoid nutrient limitation. Similar to plants raised from seeds all plants raised from cuttings, were returned to the glasshouse in mid-February 2008, blocked according to the experimental blocks of the field experiment and grown under the same conditions as described for plants grown from seeds.

MEASUREMENTS

Plant individuals were monitored daily for the onset of flowering. The following measurement and harvesting scheme was used (Table S2). The first flowering offspring of each maternal plant grown from seeds was harvested when the first flower head was completely open. The second and third offspring of each maternal plant grown from seeds and the cuttings were harvested when the first capitulum produced ripe seeds. Harvest covered a period of 5 weeks starting on 1 March 2008. All plants that did not produce any buds or flower heads were harvested on 4–5 April 2008. Leaf number, number of flower heads and the length of the longest leaf were recorded on all individuals. Flower head diameter and root biomass were only measured for the first offspring of each maternal plant grown from seeds. For the second offspring of each maternal plant grown from seeds and for the cuttings, seeds of the first capitulum that produced ripe seeds were collected and seed mass was determined. Seed mass

per plant was estimated as seed mass per flower head multiplied by the number of flower heads. Additionally, a bulk sample with 30 seeds was weighed to determine average seed mass. Seed number per plant was assessed by dividing seed mass per plant by average seed mass. The dry mass of all plant parts, i.e. shoots, roots, inflorescences and seeds was determined after drying at 70°C (48 h). In total, 3149 plants were harvested: 1174 plants raised from cuttings and 1975 plants raised from seeds.

STATISTICAL ANALYSES

Mixed-effects models with maximum likelihood (ML) estimation of fixed effects and variance components (software R, version 2.9.0, R Development Core Team 2007, http://www.R-project.org; package *lme4;* [55]) were used for data analysis because of the unbalanced design (different numbers of plots and plants for the resident and colonizer populations and seedling and cutting families) and the multiple random terms in the model [56]. Blocks of the greenhouse and blocks and plots of the biodiversity experiment were considered as random terms. Family (= maternal plant in case of seedlings) was included as an additional random term when traits were measured on several offspring per maternal plant. The fixed terms were plant origin (cuttings vs. seedlings), main selection regime (K- vs. r-selection), biodiversity selection regime (species richness of the experimental communities as log-linear term (log SR) and presence/absence of each of the four functional groups in the experimental communities) and interactions of the previous terms. These fixed terms were added stepwise to the models. Likelihood-ratio tests were applied to compare the models obtained in the different steps and to assess the significance of the fixed effects. Because the presence/absence of particular functional groups in the experimental communities had only marginal effects in this study, we removed them from the final models. In order to meet the assumptions of normality and variance homogeneity, prior to the analyses, all variables were log-transformed, except for the germination rates (arcsine square-root transformed) and the number of flower heads (not transformed). The R code with the final model structure is given in the Appendix.

Acknowledgements

We thank E.-D. Schulze, W.W. Weisser, A. Weigelt and many others for their continued support towards maintaining the Jena Experiment. Furthermore we thank all people, in particular A. Fastnacht, helping with plant cultivation and harvest of the *T. officinale*

experiment. We greatly acknowledge I. Uhlemann for information about the taxonomy and distribution of *T. officinale* in Germany.

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Table 1: Mixed-effects model analysis of characteristics measured on plants grown from cuttings or seeds of resident and colonizer populations of *T. officinale* in the Jena Experiment, 5 years after sowing.

	Germination (%)		Shoot biomass		Root biomass		Shoot:root ratio		Vegetative biomass		Reproductive biomass	
	L ratio	p	L ratio	p	L ratio	p	L ratio	p	L ratio	p	L ratio	p
Seedlings (S) vs. cuttings (C)	n.a.	n.a.	19.68	<0.001	n.a.	n.a.	n.a.	n.a.	25.73	<0.001	0.63	0.428
Disturbance (rK)	1.93	0.165	0.01	0.905	16.23	<0.001	18.79	<0.001	0.88	0.348	0.38	0.537
Species richness (log SR)	5.30	0.021	6.01	0.014	0.02	0.886	0.11	0.739	2.80	0.094	3.80	0.051
rK x log SR	1.57	0.210	2.29	0.130	0.84	0.358	0.55	0.457	1.16	0.282	3.35	0.067
S vs. C x rK	n.a.	n.a.	0.16	0.692	n.a.	n.a.	n.a.	n.a.	1.91	0.167	0.38	0.538
S vs. C x log SR	n.a.	n.a.	2.82	0.093	n.a.	n.a.	n.a.	n.a.	0.03	0.856	1.02	0.312

	Number of leaves		Maximum leaf length		Number of flower heads		Flower head diameter		Seed mass per flower head		Mean seed mass	
	L ratio	p	L ratio	p	L ratio	p	L ratio	p	L ratio	p	L ratio	p
Seedlings (S) vs. cuttings (C)	0.05	0.817	5.58	0.018	0.05	0.821	n.a.	n.a.	8.89	0.003	10.53	0.001
Disturbance (rK)	6.54	0.011	0.09	0.771	21.35	<0.001	2.67	0.102	17.48	<0.001	27.97	<0.001
Species richness (log SR)	0.23	0.629	1.11	0.291	0.58	0.448	5.87	0.015	2.85	0.091	<0.01	0.948
rK x log SR	0.86	0.354	0.52	0.473	3.55	0.060	3.19	0.074	1.17	0.279	2.97	0.085
S vs. C x rK	3.05	0.081	0.33	0.568	0.54	0.464	n.a.	n.a.	0.76	0.382	15.56	<0.001
S vs. C x log SR	2.33	0.127	1.40	0.237	0.16	0.694	n.a.	n.a.	0.05	0.823	1.33	0.249

	Seed mass per plant		Seed number per plant		First day of flowering		Day of seed maturity		Height of the flower stalk	
	L ratio	p	L ratio	p	L ratio	p	L ratio	p	L ratio	p
Seedlings (S) vs. cuttings (C)	3.86	0.049	0.71	0.400	n.a.	n.a.	1.58	0.208	0.66	0.415
Disturbance (rK)	3.83	0.050	13.22	<0.001	8.3	0.004	2.33	0.127	0.99	0.319
Species richness (log SR)	2.06	0.151	1.86	0.172	1.65	0.199	3.80	0.051	0.48	0.487
rK x log SR	5.27	0.022	0.98	0.321	0.13	0.716	0.11	0.741	0.28	0.599
S vs. C x rK	98.99	0.292	5.18	0.023	n.a.	n.a.	3.79	0.051	6.12	0.013
S vs. C x log SR	17.92	0.438	0.11	0.739	n.a.	n.a.	0.81	0.369	0.32	0.572

Note: Models were fitted by stepwise inclusion of model terms. Listed are the results of likelihood-ratio tests that were applied to assess model improvement (L ratio) and the statistical significance of these tests (p values).

Table 2: Plant characteristics averaged across all resident and colonizer populations measured in plants grown from cuttings or seedlings of *T. officinale* in the Jena Experiment, 5 years after sowing.

Measured trait	Cuttings	Seedlings
Shoot biomass (mg)	948 (± 12)	1595 (± 18)
Vegetative biomass (mg)	513 (± 7)	1162 (± 12)
Reproductive biomass (mg)	521 (± 7)	573 (± 11)
Number of leaves	11 (± 0)	11 (± 0)
Maximum leaf length (cm)	18.2 (± 0.1)	22.8 (± 0.1)
Number of flower heads	1.7 (± 0.0)	1.6 (± 0.0)
Seed mass per flower head (mg)	97.5 (± 1.1)	119.6 (± 1.5)
Mean seed mass (mg)	0.48 (± 0.00)	0.61 (± 0.01)
Seed mass per plant (mg)	192.8 (± 3.7)	246.7 (± 6.0)
Seed number per plant	467 (± 16)	414 (± 8)
Day of seed maturity	32 (± 0)	30 (± 0)
Height of the flower stalk (cm)	37.2 (± 0.3)	39.0 (± 0.4)

Values are means (± 1 SE).

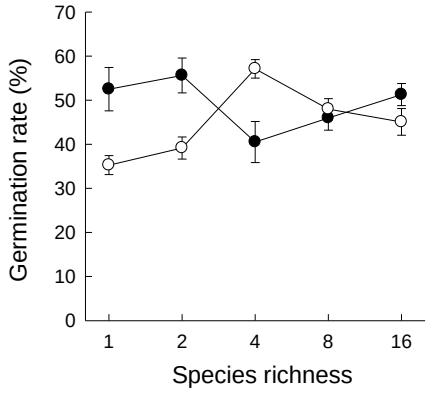

Fig. 1: Species richness effects on germination rates of seeds of *T. officinale*.Material was collected from colonizer populations (open circles) and resident populations (closed circles) in the Jena Experiment, 5 years after sowing. Values are means per species-richness level ± 1 SE.

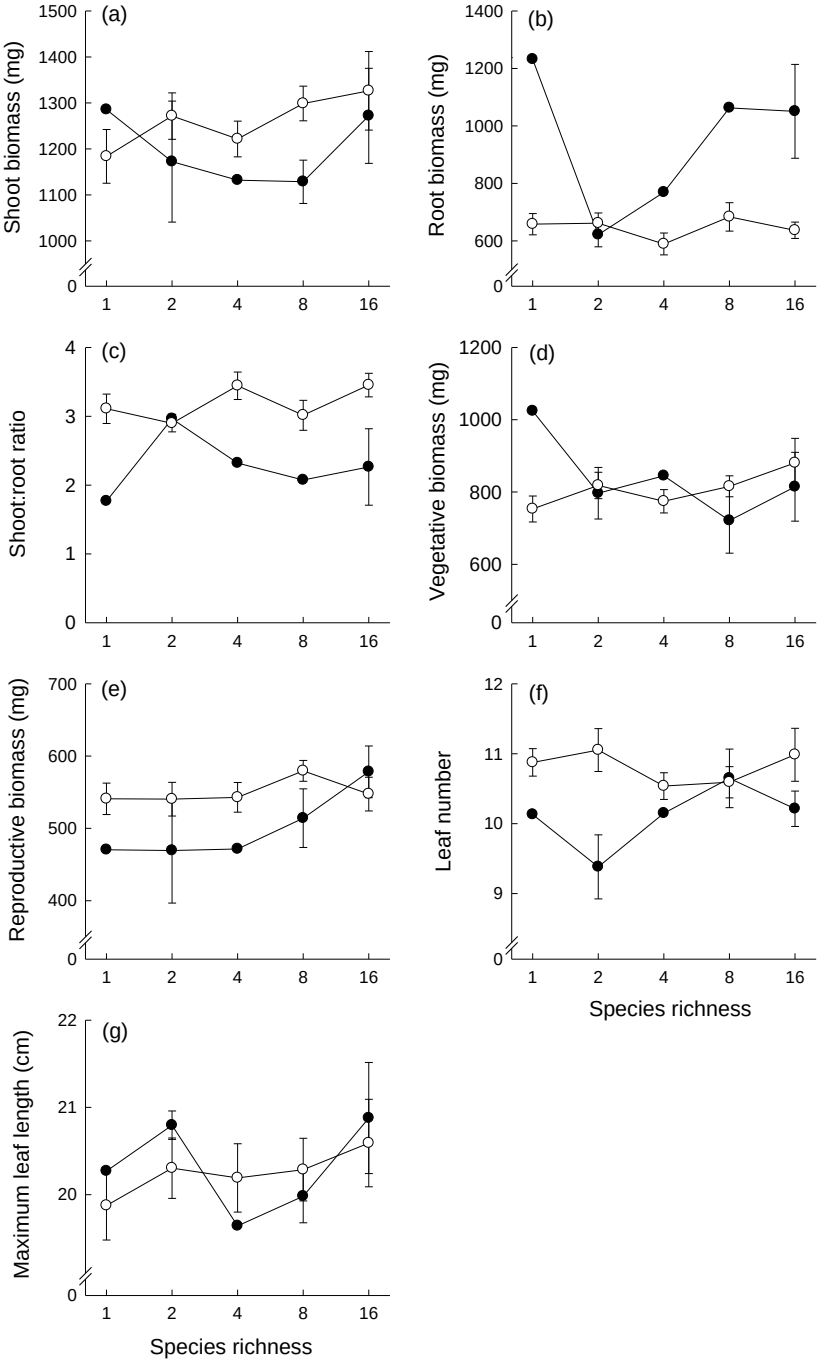

Fig. 2: Species richness effects on biomass and vegetative plant characteristics of *T. officinale*. Shoot biomass (a), root biomass (b), shoot:root ratio (c), aboveground vegetative biomass (= leaves) (d), reproductive biomass (e), leaf number (f), and maximum leaf length (g) were measured in plants grown from seeds or cuttings of colonizer populations (open circles) and resident populations (closed circles) of *T. officinale* in the Jena Experiment, 5 years after sowing. Values are means per species-richness level ± 1 SE.

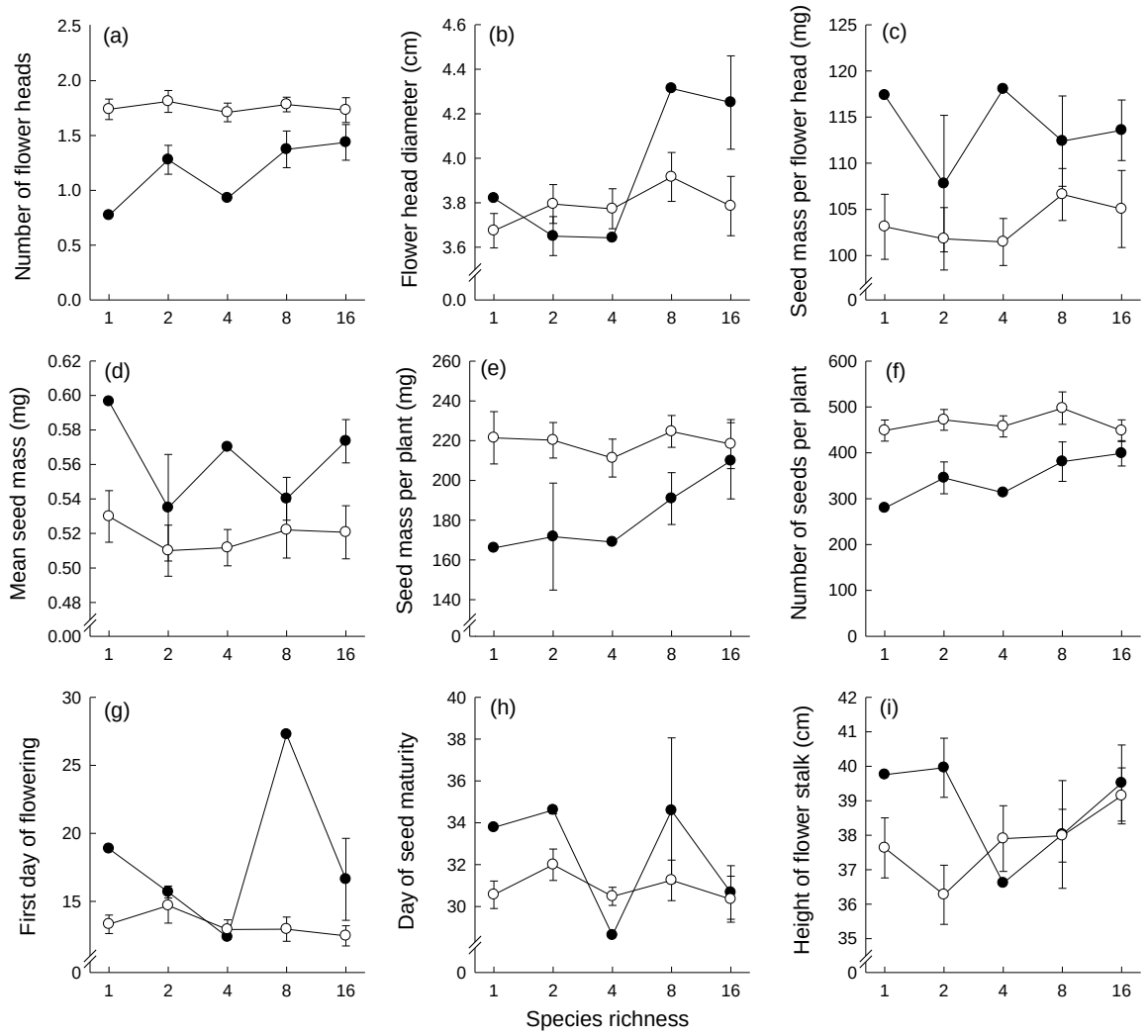

Fig. 3: Species richness effects on reproductive plant characteristics of *T. officinale*.
Flower head number (a), flower head diameter (b), seed mass per flower head (c), seed mass per plant individual (d), average seed mass (e), seed number per plant individual (f), first day of flowering (g), first day of seed maturity (h), and flower stalk length at seed maturity (i) measured in plants grown from seeds or cuttings of colonizer populations (open circles) and resident populations (closed circles) of *T. officinale* in the Jena Experiment, 5 years after sowing. Values are means per species-richness level ± 1 SE.

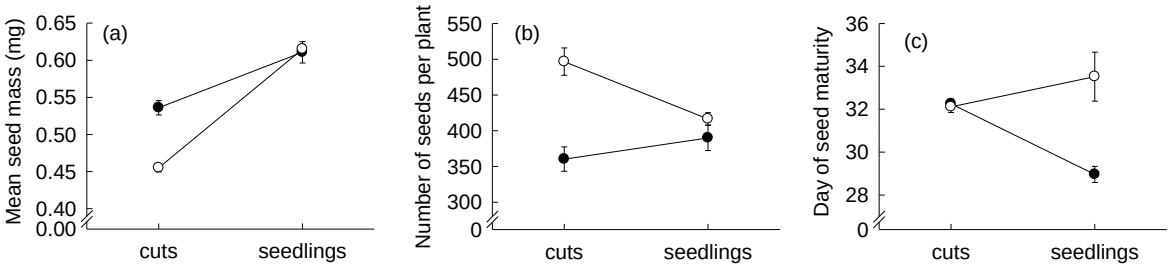

Fig. 4: Differences between cuttings and plants grown from seeds in reproductive plant characteristics of *T. officinale*. Mean seed mass (a), number of seeds per plant (b), and day of seed maturity (c) measured in plants grown from seeds and from cuttings of colonizer populations (open circles) and resident populations (closed circles) of *T. officinale* in the Jena Experiment, 5 years after sowing. Values are means across all resident and colonizer populations for cuttings and plants grown from seeds ± 1 SE.

Supporting Information

Table S1: Origins of plants grown from cuttings and seeds of *Taraxacum officinale* from experimental plots sown with a different number of species.

		Plants grown from seeds		Plants grown from cuttings	
		Resident populations	Colonizer populations	Resident populations	Colonizer populations
Species richness	1	1	12	1	16
	2	2	10	2	14
	4	1	11	1	15
	8	1	11	4	12
	16	3	8	5	9

In 13 plots the species was sown as resident of the experimental community (resident populations, K-selection regime), in all other plots it was colonizing and continuously removed by weeding (colonizer populations, r-selection regime). Fewer experimental plots were used for plants grown from seeds because plants of one experimental block were destroyed by mice.

Table S2: Measured traits of plants grown from seeds or cuttings.

Traits	Grown from seeds			Grown from cuttings
	Offspring 1	Offspring 2	Offspring 3	
Shoot biomass (total) (mg)	×	×	×	×
Root biomass (mg)	×	n.a.	n.a.	n.a.
Shoot:root ratio	×	n.a.	n.a.	n.a.
Vegetative biomass (leaves) (mg)	×	×	×	×
Reproductive biomass (stalk, flowers, seeds, buds) (mg)	×	×	×	×
Number of leaves	×	×	×	×
Length of the longest leaf (cm)	×	×	×	×
Number of flower heads	×	×	×	×
Flower head diameter (cm)	×	n.a.	n.a.	n.a.
Mean seed mass (mg)	n.a.	×	n.a.	×
Seed mass per flower head (mg)	n.a.	×	n.a.	×
Seed mass per plant (mg)	n.a.	×	n.a.	x
Seed number per plant	n.a.	×	n.a.	×
First day of flowering*	×	n.a.	n.a.	n.a.
Day of seed maturity*	n.a.	×	×	×
Height of flower stalk at seed maturity (cm)	n.a.	×	×	×

Note: n.a. = not measured

* day from 01.03.2008 to 05.04.2008.

The R code for statistical analysis with mixed-effects models

We used R 2.9.0 (R Development Core Team 2007) and the R package *lme4* [55] for data analyses.

Abbreviations of variable names are:

Glasshouseblock	Experimental blocks in the glasshouse for cultivation of offspring from the biodiversity experiment
Fieldblock	Experimental blocks of the biodiversity experiment
Plot	Experimental plots of the biodiversity experiment
Family	Identity of maternal plants of seedlings
Cultivation	Plants origin (seedling vs. cutting)
rK	Selection regime (r vs. K-selection)
sowndiv	Species richness of the experimental plots of the biodiversity experiment

R code:

```
sg0<-
lmer(X~1+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg1<-
lmer(X~1+Cultivation+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg2<-
lmer(X~1+Cultivation+rK+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg3<-
lmer(X~1+Cultivation+rK+log(sowndiv)+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg4<-
lmer(X~1+Cultivation+rK+log(sowndiv)+rK:log(sowndiv)+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg5<-
lmer(X~1+Cultivation+rK+log(sowndiv)+rK:log(sowndiv)+Cultivation:rK+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
sg6<-
lmer(X~1+Cultivation+rK+log(sowndiv)+rK:log(sowndiv)+Cultivation:rK+Cultivation:log(sowndiv)+(1|Glasshouseblock)+(1|Fieldblock)+(1|Fieldblock:Plot)+(1|Fieldblock:Plot:Family),REML=F)
anova(sg0,sg1,sg2,sg3,sg4,sg5,sg6)
```

General Discussion

Biodiversity experiments have repeatedly shown that increasing plant diversity has positive effects on a number of ecosystem processes such as aboveground productivity and nutrient cycling (Hooper *et al.* 2005; Balvanera *et al.* 2006). However, in spite of these positive relationships between species richness and aboveground productivity, responses of individual species to altered community diversity vary greatly (Dimitrakopoulos & Schmid 2004; Roscher *et al.* 2007; Thein *et al.* 2008; Marquard *et al.* 2009). So far, these differential responses at the community level and at the species level are not mechanistically understood.

The central focus of my work carried out in the framework of the Jena Experiment, a large-scale long-term biodiversity experiment, was to explore the effects of increasing plant diversity on the performance of forb species. Particularly, the effects of increasing species richness of the experimental grasslands on trait variation of forb species were studied with the aim to get insight to which extent responses of forb species to increasing plant diversity are due to phenotypic plasticity or genetic variation of these species. Therefore, (1) I studied the performance and functional trait variation of 27 forb species to increasing species richness (chapter 1), and (2) set up two different experiments to differentiate between plant diversity effects on phenotypic plasticity and genetic variation in selected forb species. Firstly, I performed a reciprocal transplant-replant experiment based on seed families of five forb species collected in monocultures and 60-species mixtures five years after the establishment of the Jena Experiment to analyze whether the observed trait variation between plants growing in communities of different plant diversity are caused by phenotypic plasticity or genotypic variation of these species (chapter 2). Secondly, I investigated whether plants of *Taraxacum officinale,* a forb species which is included in the Jena experimental species pool, but at the same time occurs as the most colonizer in plots where it was not sown, after 5 years of different selection regimes created through weeding, developed trait differences when grown in a common environment (chapter 3). By synthesizing the chapters of my thesis, I exemplarily may demonstrate some relationships between species diversity, environmentally induced trait variation and genetic differentiation.

The positive effect of plant diversity on ecosystem processes (Hooper *et al.* 2005) such as primary productivity (Hector *et al.* 1999; Tilman *et al.* 2001; Roscher *et al.* 2005), ecosystem stability (Loreau 2000) and nutrient cycling (Naeem *et al.* 1994; Hooper & Vitousek 1998) is based on the number, identity and differences of species within this community (Balvanera *et al.* 2006) and the extremely variability of many traits within single species (Grassein *et al.* 2010). This variability of functional traits allows plant species to cope with spatial and/or

temporal environmental heterogeneity and could also be shown for the studied forb species in the Jena Experiment (chapter 1). A niche separation through functional differences among forb species is important to receive species richness in the small experimental area. Differences in the expression of different trait combinations indicated species-specific strategies to obtain the species-specific amount of light and nutrient for growth and reproduction. The consideration of single species is important to examine the assembly of plant communities and ecosystem processes in species-rich plant communities. This variability can be caused by phenotypic plasticity and /or genotypic variability. However, so far only few empirical studies (Vellend and Geber 2005; Fridley et al. 2007; Roscher et al. 2008; Silvertown et al. 2009) documented the relationships between species richness (especially experimental richness gradients), phenotypic plasticity and genetic variability.

Generally, I observed that species richness was related to trait variation in the Jena Experiment. Increasing species richness influences light and nutrient availability in the experimental plots (e.g. Dassler et al. 2008; Lorentzen et al. 2008; Mulder et al. 2002, Hector et al. 2007). Light and nutrient availability are important drivers which may induce variation in morphological and physiological traits of forb species to optimize resource acquisition and to adjust to different resource availability dependent on plant neighbourhood (chapter 1). The investigated forb species, which represent different growth forms and vary in their phylogenetic relatedness had unique combinations in their functional traits and a highly variable correlation structure among these traits, which are a prerequisite for complementarity among them. Although my results support the conclusion derived from earlier experiments (Reich *et al.* 2003) and from data of the functional group of legumes and grasses (Roscher *et al.* 2011; Gubsch *et al.* 2011) in the Jena experiment, I found that that main ecological strategies are robust across different forb species. Particularly physiological traits associated with nitrogen acquisition and their variation in response to increasing plant diversity was phylogenetically conserved to some degree in the investigated forb species. However, co-occurrence with an increasing number of other plants (i.e. species richness) or neighboring species with particular traits (i.e. effects of legumes) had stronger effects than phylogenetic relatedness on functional trait expression of the studied forb species. Thus, phenotypic integration is probably a more powerful driver of variation in single traits than effects of shared ancestry in the investigated forb species. However, phylogenetically closely related species respond with similarly directed trait variation in traits associated with light capture compared to distantly related species. Likely, the obtained results of the phylogenetic

relatedness differ between natural plant communities and my results of the in the Jena Experiment where artificial plant communities sustained by weeding.

The design of the Jena Experiment allows to identify the dimension of trait variation in plants grown in different communities, possessing different growth forms and originating from different phylogenetic groups which was rarely possible in this complexity in previous studies. However, the Jena Experiment has also some dearth for studying trait variation in single species and to differentiate this variation into phenotypic plasticity and genotypic variability. The Jena Experiment was planned as a biodiversity experiment to explore the effects of species richness and functional group richness on ecosystem processes, and therefore the experimental design carefully combines different levels of species richness with different functional group compositions (Roscher *et al.* 2004). The experiment was initially not designed to study the responses of single species which were randomly chosen for the replicates at each level of species and functional group richness. However, in the Jena Experiment all species growing in multi-species assemblages were also sown as monocultures at the same experimental field and in replicated 60-species mixtures. Based on my results on the variation of plant traits of forb species in response to increasing plant diversity, I initiated two specific sub-experiments to investigate the effects of plant diversity on single plant species (chapters 2 and 3). Previous research of individual species in experimental grasslands has shown that species may possess strategies to maintain or even increase their performance in communities of different plant diversity (Dassler *et al.* 2008; Thein *et al.* 2008; Marquard *et al.* 2009). However, it remained unclear whether the differential responses of individual species in response to increasing plant diversity (chapter 1) are based on phenotypic variation or genetic differentiation within single species.

In chapter 2, my results based on five forb species show that most variation among the plants growing in different environments (monocultures versus 60-species mixtures) was attributable to environmentally-induced phenotypic plasticity. For the investigated plant species, five growing seasons in monocultures or mixtures were not sufficient to clearly demonstrate the selection of genotypes specifically adapted to monocultures or mixtures. Results from further diversity experiments indicated that the time scale is important. It is assumed that in experimental grassland plant interactions change and develop after the start of the experiment (Van Ruijven & Berendse 2009). It is known that a single species (or genotypes) are unlikely to be both, good competitors and good colonizers (Fakheran *et al.* 2010). Thus, it is likely that genotypic composition of plant populations changed over time. Compared to Fakheran et al. (2010) my experiment was set up to test for monoculture vs. mixture genotype selection and

not to test for colonizer vs. competition genotypes and its special traits. Obviously, two to not more than four generations of the used experimental species was a short time span to find genotypic differences in the investigated traits of the 5 forb species. However, the controlled reciprocal transplant experiment gave first evidence that monoculture and mixture environments may lead to genetic differentiation in plant species and a local adaptation to their biotic environment, which implies the evolution of monoculture and mixture genotypes.

Furthermore, I analyzed intraspecific differences in the model species *Taraxacum officinale* (chapter 3). We created r- and K-selection regimes by weeding (density-independent mortality, r-selection) and increasing interspecific competition along the species richness gradient (K-selection) within the experimental design of the Jena experiment. Based on a common garden experiment with offspring raised from cuttings or seeds of these manipulated populations, I present in chapter 3 for a number of traits characterizing biomass allocation and reproductive output that density-independent mortality (r-selection) caused different adaptive responses than density-dependent mortality (K-selection). These results support former studies (e.g. Hughes *et al.* 2008; Kotowska *et al.* 2010) that plant genotypic diversity may have consequences for ecosystem function. The different genotypes of *Taraxacum officinale* in the Jena Experiment may alter the functioning of several fundamental ecological processes based on traits of competitive ability or reproduction. Although, this variation within a single species may be smaller than that among different species, it may nevertheless be large enough to impact ecosystem functioning.

In conclusion, this thesis presents several results regarding the question about the relevance of examination of single species in biodiversity experiments. Despite or gerade hence the evidence that multi-species rather than single species effects mainly generate positive biodiversity effects, these effects are only possible by the different species-specific strategies in resource acquisition and use and the variability of trait expression in a species richness gradient. The findings are relevant with regard to the future functionality of ecosystems. Knowledge about phenotypic variability in response to species richness is important for understanding trait-mediated species interactions and to predict species responses to global change (Potvin & Tousignant 1996). Empirical studies have demonstrated that plant genetic diversity improves ecosystem resistance to exotic plant invasions (Crutsinger *et al.* 2008), enhance plant community resistance to extreme climatic events (Reusch *et al.* 2005) and land use as grazing (Hughes & Stachowicz 2004), accelerate litter decomposition rates (Schweitzer *et al.* 2005) and maintain long-term species diversity (Booth & Grime 2003; Vellend 2006).

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Summary

Summary

In the past, numerous experiments in grassland ecosystems have analyzed the positive effects of biodiversity on several ecosystem processes, e.g. primary productivity or nutrient cycling. These positive biodiversity-ecosystem functioning relationships were attributed to complementarity among species. The biological mechanisms subsumed under the term "complementarity" are not well understood, as we are only beginning to understand the role of single species or species interactions in ecosystems of different plant diversity. I used data from a large biodiversity experiment (Jena Experiment) to examine mechanisms underlying plant community assembly and especially the role of single species within these communities.

In chapter 1, I assessed the variation of a number of functional traits measured on aboveground plant organs of 27 different forb species in response to biodiversity (i.e. in particular species richness and legume presence) and related trait variation to phylogenetic differences among species and their different growth-forms. I observed that plant responses to changes in light and nutrient availability attributable to differences in species richness and legume presence are robust across different forb species. However, I found evidence for species-specific strategies in various functional trait combinations and variable correlation structures of these traits, which are the base for niche partitioning among forb species and may allow for increasing complementarity in more diverse systems. I found that phylogenetically more closely related species are likely to express more similar strategies when competing for light than less related species. However, different growth forms had significant impacts on variation in traits associated with nitrogen acquisition and use in the vegetative stage of the studied forb species.

In chapter 2, I examined whether the variation in plant individual performance to increased species richness (monoculture vs. 60 species mixture) is due to phenotypic plasticity or genetic variation. I performed a reciprocal transplant-replant experiment with the offspring of seed families of five forb species, which I collected in species monocultures and mixtures of 60 species. I observed differences in plant survival, performance in terms of aboveground biomass and investment into reproduction between offspring of plants growing in an actual environment of different plant diversity. Most variation among transplants growing in different environments was attributable to environmentally-induced phenotypic plasticity through differences in light and nutrient availability and herbivore pressure. I found no evidence for local genetic adaptation to monocultures or mixtures after five years of selection in different environments. However, four of the five study species showed a genetic

differentiation between the two different selection regimes indicating a tendency to local adaptation.

In chapter 3, I used the model species *Taraxacum officinale*, belonging to the experimental species pool but being also the major colonizer species in the Jena Experiment, to examine intraspecific differentiation by creating different selection regimes by weeding (density-independent mortality, r-selection) and increasing interspecific competition along a species richness gradient (density-dependent mortality, K-selection). I hypothesized that plants after five years of different selection regimes develop traits expected under r and K selection in a common environment. I observed the expected assumption in *Taraxacum officinale* plants, which suggests that the different selection pressures may result in a rapid genetic differentiation in this apomictic species. However, my experimental design did not allow conclusions about the mechanism behind this observed genetic differentiation. From these results I concluded that intraspecific plant genetic diversity may play an important role in grassland plant diversity and is important to consideredin actual biodiversity research and conservation discussion.

Zusammenfassung

Zahlreiche Experimente in Grasland-Ökosystemen haben positive Effekte der Biodiversität auf Ökosystemprozesse wie Primärproduktion oder Nährstoffkreisläufe gefunden. Diese positiven Effekte der Biodiversität auf das Funktionieren der Ökosysteme wird der Komplementarität zwischen den Arten zugeschrieben. Die biologischen Mechanismen die sich unter dem Begriff Komplementarität zusammenfassen lassen, sind jedoch noch nicht gut verstanden, so ist zum Beispiel die Rolle einzelner Arten in einem Ökosystem oft unklar. Für die vorliegende Arbeit nutzte ich Daten aus einem großflächigen Grünland-Biodiversitätsexperiment (Jena Experiment) um Mechanismen zu untersuchen, denen die Strukturierung von Pflanzengemeinschaften unterliegt. Dabei versuchte ich auch die Rolle einzelner Arten und ihre Beziehung untereinander zu verstehen.

In Kapitel 1 untersuchte ich die Reaktion verschiedener Kräuterarten auf eine steigende pflanzliche Diversität, besondere Beachtung fad dabei die pflanzliche Artenzahl und die Anwesenheit von Leguminosen. Die pflanzliche Reaktion wurde anhand einer Reihe von Merkmalen der oberirdischen Pflanzenorgane gemessen. Anschließend analysierte ich, ob diese Variation von der phylogenetischen Verwandtschaft der einzelnen Arten untereinander und/oder von ihren verschiedenen Wuchsformen abhängig ist. Die messbare pflanzliche Reaktion der untersuchten Arten ist bedingt durch verschiedenen Licht- und Nährstoffverhältnissen in den experimentellen Plots unterschiedlicher Artendiversität und robust in allen untersuchten Kräuterarten. Jedoch fand ich auch Belege dafür, dass es artspezifische Strategien in den vielfältigen Merkmalskombinationen und den variablen Korrelationsstrukturen dieser Merkmale gibt, welche die Basis für die Nischenaufteilung zwischen den Kräutern sind. Diese Nischenaufteilung ermöglicht eine höhere Komplementarität in stärker diversen Systemen. Außerdem fand ich Hinweise darauf, dass phylogenetisch nahverwandte Arten ähnlichere Strategien unter Lichtkonkurrenz entwickeln als weniger nahverwandte Arten. Für Merkmale der Stickstoffaneignung und –nutzung im vegetativen Stadium der Kräuter hatte die Wuchsform einen signifikanten Einfluss, während die phylogenetische Verwandtschaft keine signifikante Rolle spielte.

Im Kapitel 2 analysierte ich, ob die Ausprägung von Merkmalen bei Individuen aus Monokulturen und 60 Arten-Mischungen auf phänotypische Plastizität oder genetische Variation zurückzuführen ist. Ich führte ein reziprokes Verpflanzungsexperiment mit den Nachkommen von Samenfamilien, die ich für 5 krautige Arten in ihren Monokulturen und den60-Arten-Mischungen sammelte, durch. Die Nachkommen der Pflanzen unterschieden sich in ihrer Überlebensrate, der oberirdischen Biomasse und Investition in ihre Reproduktion

in Abhängigkeit von ihrem aktuellen Wuchsort. Die größte Variation zwischen den Pflanzen, welche in diesen unterschiedlichen Umwelten aufwuchsen, war auf umweltinduzierte phänotypische Plastizität durch Unterschiede in Licht- und Nährstoffverfügbarkeit sowie verschiedene Grade von Herbivorie zurückzuführen. Ich konnte nach fünf Jahren Selektion keine Hinweise auf eine genetische Adaptation von Monokultur- oder Mischungsgenotypen finden. Jedoch zeigten vier der fünf untersuchten Arten genetische Unterschiede zwischen den beiden verschiedenen Wuchsorten/ Selektionsregimen, die somit in geringem Umfang auf lokale Adaptation hinweisen.

In Kapitel 3 nutzte ich die Modellart *Taraxacum officinale*, die einerseits Zielart, andererseits aber auch einer der stärksten Invasoren/Unkräuter im Jena Experiment ist, um innerartliche Unterschiede in dieser Art zu untersuchen. Dabei wurde der unterschiedliche Selektionsdruck, dem *Taraxacum officinale* im Jena Experiment ausgesetzt ist, genutzt. Dieser Selektionsdruck wurde zum Einen durch das Jäten (dichte-unabhängige Mortalität, r-Selektion) zum Anderen durch erhöhte interspezifische Konkurrenz entlang des Diversitätsgradienten (dichte-abhängige Mortalität, K-Selektion) erreicht. Mein Ziel war die Klärung, ob Pflanzen, die in gleicher Umgebung aufwachsen, Merkmale, die auf die r- und K-Selektion zurückzuführen sind, entwickeln. Diese Erwartungen konnte ich durch das Experiment bestätigen. Ich schlussfolgere daraus, dass der vorherrschende Selektionsdruck zu einer schnellen genetischen Differenzierung der apomiktischen Art *Taraxacum officinale* führt. Die Ursachen die zur Differenzierung der Art führen, konnte ich in der vorliegenden Arbeit jedoch nicht klären. Ich schliesse jedoch aus diesen Ergebnissen, das die innerartliche genetische Diversität bei Pflanzen eine wichtige Rolle in Wiesenökosystemen spielt und diese deshalb in der aktuellen Biodiversitäts- und Naturschutzdebatte mit aufgegriffen werden sollte.

Acknowledgements

I would like to thank a number of people. First, I thank Bernhard Schmid and Christiane Roscher for supervising this thesis. Bernhard is a great supervisor for my stil to work. I thank him for giving me a great freedom for my work but also fast and uncomplicated help at all research and administrative questions. Thanks, for giving me the opportunity to work on interesting questions in ecology and for employing me at the IfU after my contract in Jena had ended.

Thanks Christiane for the excellent guidance of field work, statistical analyzing, paper writing, proof reading and to find always time for answers and help. It is really comfortable to have a supervisor you can ask all the time all research and administrative question and get all the time fast and practical answers.

I thank E.-D. Schulze for giving me the chance to start this PhD project and providing me an office and resources at the Max-Planck Institute for Biogeochemistry in Jena.

I would like thank Jens Schumacher for the excellent statistical consultations (with providing of tea and toys) and to find always time to think about ways to get the analyses which I or Bernhard would have been.

Furthermore, I thank the remaining members of my PhD committee Markus Fischer and Andy Hector.

I thank Stefan Michalsky for the fast and uncomplicated help with the phylogenetic tree and questions arising from it.

Very special thank to the members of IfU for help with the administrative stuff at all time and especially I thank Isabel for her help and to took the telephone to get a faster answer.

Very special thanks go to Agnes Fastnacht to providing me place in the greenhouse, help with cultivation and harvest of my plants and have a nice time at this place. I thank Iris Kuhlmann, Heike Geilmann and Ines Hilke for technical support and chemical analyzing in the MPI and the gardeners of the Jena Experiment as well as numerous student helpers (especial Uta and Carolina for additional discussions) for their work at the field site and in the lab.

Special thanks to Alex Weigelt to coordinated the whole project, all questions and wishes in terms of plot using. Thanks for the nice time with my colleagues of the Jena Experiment, especially Tanja, Lisa, Jana and Marlen for being not only colleagues but sharing the same situation in the same project and would be friends during this time. Tanja thanks for your help

and time of babysitting and Jana, Marlen and Alex to provide me a place to sleep in Zürich and have a nice time there.

Additional thanks to Tina Nolte for proofreading.

Thanks all of my friends for their continuous mental support.

Ich danke meiner Familie für all ihre Unterstützung und vor allem meiner Oma nicht nur für das Kinderhüten.

THANKS Justus

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